Talanta 111 (2013) 62–68

Contents lists available at SciVerse ScienceDirect

Talanta

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

Detection and discrimination of alpha-fetoprotein with a label-free electrochemical impedance spectroscopy biosensor array based on lectin functionalized carbon nanotubes

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ARTICLE INFO

Article history:
Received 20 October 2012
Received in revised form
23 January 2013
Accepted 30 January 2013
Available online 13 February 2013

Keywords:
Biosensor
Array
Electrochemical impedance spectroscopy
Alpha-fetoprotein
Lectin

ABSTRACT

A label-free electrochemical impedance spectroscopy (EIS) biosensor for the sensitive determination and discrimination of alpha-fetoprotein (AFP) was developed by employing wheat-germ agglutinin (WGA) lectin as molecular recognition element. The EIS biosensor was fabricated by adsorbing carboxyl-functionalized single-wall carbon nanotubes (SWNTs) onto a screen-printed carbon electrode (SPCE) and subsequently covalently coupling WGA onto the surface of the SWNTs-modified electrode. Upon binding of AFP to the biosensor, the electron transfer resistance was increased and the increase in the electron transfer resistance was linearly proportional to the logarithm of the concentration of AFP in the range from 1 to 100 ng/L with a detection limit of 0.1 ng/L. It was found that the employment of SWNTs as immobilization platform could reduce the background and enhance the EIS response. Moreover, the lectin-based biosensor array fabricated with different lectins was used to evaluate the glycan expression of AFP N-glycan and discriminate AFP between healthy and cancer patients serum samples. This work demonstrates that the employment of carbon nanotubes as immobilization platform and lectin as molecular recognition element in biosensor array is a promising approach for the determination and discrimination of glycoproteins for cancer diagnosis. The strategy proposed in this work could further be used for high-throughput, label-free profiling of the glycan expression of cancer-related glycoproteins and to develop methods for cancer diagnosis in the early stages.

1. Introduction

The development of the highly sensitive and high-throughout method for the detection of tumor markers is one of the most rapidly growing research areas in clinical tests for early discovery of cancer [1,2]. Alpha-fetoprotein (AFP), with a molecular weight of 70 kDa, has been known as a reliable biomarker for hepatocellular carcinoma [3]. A variety of methods have been developed to determine AFP, such as fluorescence immunoassay [4], electrogenerated chemiluminescence (ECL) immunoassay [5,6] and electrochemical immunoassay [7–13]. Despite the extensive development of these methods, each design has its own advantages and disadvantages. Among them, electrochemical immunoassay has received much attention due to its unique advantages, such as a low detection limit, small analyte volume, simple instrumentation, and minimal manipulation [14].

Choice of molecular recognition elements is of great importance for the fabrication of electrochemical bioassay because molecular recognition element onto electrode not only produces a functionalized sensing interface but also determines the sensitivity and selectivity of the bioassay [15]. Lectins, a group of proteins extracted from plants or animals, can strongly bind to specific carbohydrate moieties on the surface of glycoproteins [16] and thus are particularly interesting candidates as molecular recognition elements because of their ease of production and intrinsic stability. Compared with antibody-based immunoassay, the lectin-based bioassay could detect not only the content but also the aberrant glycosylation of the tumor markers, and could increase the specificity for cancer diagnosis because aberrant glycosylation is a fundamental characteristic of progression of cancer [17,18]. For example, the detection of the sialylated AFP, associated with hepatocellular carcinoma and other benign liver diseases, could be used to increase the specificity for cancer diagnosis.

Extensive efforts have been devoted to the development of electrochemical lectin-based immunoassays for the determination of the proteins and for studying the interactions between lectins and

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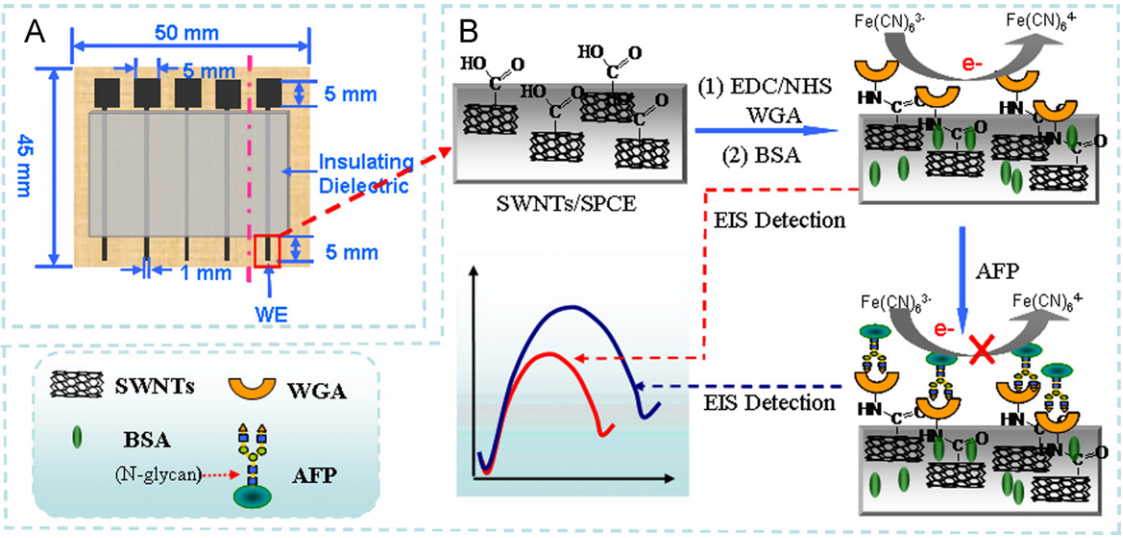
http://dx.doi.org/10.1016/j.talanta.2013.01.060



Scheme 1. Schematic representation of the base electrode array (A), fabrication of WGA-based biosensor and detection of AFP (B). (For interpretation of the references to color in this scheme, the reader is referred to the web version of this article.)

carbohydrates on tumor markers [17–20]. However, 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 impedance spectroscopy (EIS) technique offers several advantages such as simplicity, high sensitivity and serving as an elegant way to interface biorecognition events and signal transduction [21,22]. Label-free electrochemical lectin-based immunoassays for the detection of glycoproteins have also been reported [23–25]. Oliveira et al. 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 [23]. Belle et al. developed an EIS label-free, rapid 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 [24]. However, the sensitivity of these methods is limited.

The aim of this work is to develop a simple and sensitive biosensor array for the detection of AFP and evaluation of AFP N-glycan. Firstly, a label-free EIS biosensor was designed by employing wheat-germ agglutinin (WGA) lectin as molecular recognition element and carboxyl-functionalized SWNTs as amplification platform. Single-walled carbon nanotubes (SWNTs) have emerged as a very promising new class in designing novel biosensing devices due to their high conductance, tensile strength, and chemical stability [26,27]. As shown in Scheme 1, the WGA-based biosensor was fabricated by adsorbing carboxyl-functionalised SWNTs onto a screen-printed carbon electrode (SPCE) and further covalently coupling WGA onto the surface of the SWNTs-modified electrode. Upon binding of AFP, the WGA-based biosensor produces an increased EIS response that is directly proportional to the concentration of AFP. The characteristics and analytical performance of the WGA-based biosensor for the detection of AFP are reported. Moreover, a lectin-based biosensors array fabricated with different lectins preliminarily evaluate the glycan expression of AFP N-glycan and discriminate AFP between healthy and cancer patients serum samples.

2. Experimental

2.1. Reagents and apparatus

Lectins including wheat-germ agglutinin (WGA) from *Triticum vulgaris* (wheat), *Lens culinaris* agglutinin (LCA), concanavalin A (Con A), type IV from *Canvalia ensiformis* seeds, *S. nigra* agglutinin (SNA), and *Datura stramonium* agglutinin (*jimson weed, thorn apple*) (DSA), in addition to N-acetyl-glucosamine (GlcNAc), glucose, rhamnose, N-(3-dimethylamino-propy)-N′-ethylcarbodimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) and N,N-dimethylformamide (DMF) were purchased from Sigma-Aldrich (USA). Human alpha-fetoprotein (AFP, M803209) was obtained from Fitzgerald Industries International, Inc. (USA). Bovine serum albumin (BSA) and human serum albumin (HSA) were obtained from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (China). carboxyl-functionalized SWNTs (–COOH content 2.73 wt%) were obtained from Shenzhen Nanotech Port Co. Ltd. (China). Electrodag 423SS (carbon ink) and Electrodag 452SS (insulating dielectric oil) were obtained from Acheson Henkel Corporation (USA). An epoxy substrate (0.5 mm thickness) was used as a base substrate of the printed electrode.

Lectin solutions were prepared in 10 mM phosphate buffer saline (PBS, pH 7.4, 10 mM Na_2HPO_4/NaH_2PO_4 and 100 mM NaCl). AFP and GlcNAc solutions were prepared in 10 mM PBS containing 1 mM $CaCl_2$ and 1 mM $MnCl_2$. Ultra-pure water (18.2 MΩ cm) from a water production device (Milli-Q, Millipore) was used in all experiments. All other reagents were of analytical grade. Clinical serum samples were a gift from Shaanxi Cancer Hospital.

A CHI-660 electrochemical workstation (Chenhua Instruments Co., Shanghai, China) was used for the electrochemical measurements. All electrochemical experiments were performed using a conventional three-electrode system with a fabricated biosensor array or an SPCE as the working electrode, a platinum wire as the counter electrode and an Ag/AgCl (sat. KCl) as the reference electrode. All potentials are reported with respect to the reference electrode.

An atomic force microscope (AFM, SPM-9500J3, SHIMADZU Corporation, Japan) was used in contact mode to monitor the topography of the lectin-modified surfaces. The data were analyzed by IP (Thermomicroscope proscan image processing software version 2.1) and SPMLab NT 6.0.2 (Veeco, USA).

2.2. Fabrication of the biosensor

A base electrode array consisting of five working SPCE was fabricated by screen-printing technology according to Ref. [28] (Scheme 1A) [28]. An individual electrode was exactly cut from the epoxy substrate to get SPCE (as shown in Scheme 1, red dashed

line). 10 μL of SWNTs suspension, prepared by ultrasonically dispersing 10.0 mg of SWNTs in 10 mL of DMF, was drop-coated onto the surface of the SPCE and dried in air. To activate the SWNTs, 100 mL of freshly prepared solution containing 2 mg/mL EDC and 5 mg/mL NHS was dropped onto SWNTs/SPCE for 30 min. 100 μL of 1 mg/mL WGA solution was then dropped onto the activated SWNTs/SPCE for 1 h at ~25 °C. Finally, 100 μL of 1% BSA was used to block the surface active sites of the SWNTs/SPCE for 30 min. After each step, the electrode was rinsed thoroughly with washing buffer to remove adsorption components. The WGA-based biosensor (WGA/SWNTs/SPCE) was obtained and stored at 4 °C in the dark.

A lectin-based biosensor array was fabricated with different lectins, including WGA, LCA, Con A, SNA and DSA, which were prepared by immobilizing each lectin onto different SPCE surface . The procedure was same as for WGA-based biosensor except that 1 mg/mL LCA, 1 mg/mL Con A, 1 mg/mL SNA and 1 mg/mL DSA were used for other four lectin biosensors.

As control, lectin-based biosensor without SWNTs (WGA/SPCE without SWNTs) was fabricated by dropping 100 μL of 1 mg/mL WGA onto the SPCE surface for 1 h then and blocking with 100 μL of 1% BSA for 30 min at ~25 °C. After each step, the electrode was rinsed thoroughly with washing buffer to remove adsorption components.

2.3. Electrochemical measurements

The fabricated biosensor or biosensor array were immersed in 100 μL of 10 mM phosphate buffer saline (PBS, pH 7.4, 10 mM Na_2HPO_4/NaH_2PO_4 and 100 mM NaCl) containing different concentrations of AFP or serum samples for 60 min and then washed with 10 mM PBS. Electrochemical measurements were carried out in 3 mL of 10 mM PBS containing 5 mM $K_4[Fe(CN)_6]$ and 5 mM $K_3[Fe(CN)_6]$ at the equilibrium potential of $[Fe(CN)_6]^{3-/4-}$ (0.24 V vs. Ag/AgCl) with a 5 mV sinusoidal excitation amplitude. The EIS was recorded within a frequency range from 100 kHz to 0.1 Hz with a sampling rate of 12 points per decade. The concentration of AFP 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 AFP, respectively. All electrochemical experiments were carried out at room temperature ($25 \pm 1^{\circ}$ C).

3. Results and discussion

3.1. Fabrication and characterization of WGA-based biosensor

The different electrodes in the fabrication of the WGA-based biosensor were characterized by cyclic voltammetry and electrochemical impedance spectrometry in the presence of the ferri/ferrocyanide redox couple as redox probe (see Fig. 1A and B). The result showed that the electron-transfer resistance (Fig. 1A,a, $R_{et,}=0.35$ kΩ) at a SWNTs/SPCE was smaller than that at a bare SPCE (Fig. 1B,a, $R_{et}=9.06$ kΩ). This indicates that SWNTs can increase the electron transfer. The R_{et} at WGA modified electrode greatly increased to 6.34 kΩ (Fig. 1A,b), indicating that the WGA was immobilized onto SWNTs/SPCE. After blocking with BSA, the electron transfer resistances increased to 8.08 kΩ (Fig. 1A,c). The R_{et} continued to increase to 44.50 kΩ (Fig. 1A,d) after 1 ng/L AFP incubating with WGA immobilized on the surface of SPCE. 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 WGA is modified onto a SWNTs platform and WGA can specifically recognize the N-glycan on AFP [29].

As a control, a WGA/SPCE without SWNTs was fabricated to illustrate the amplification effect of SWNTs. As shown in Fig.1B,

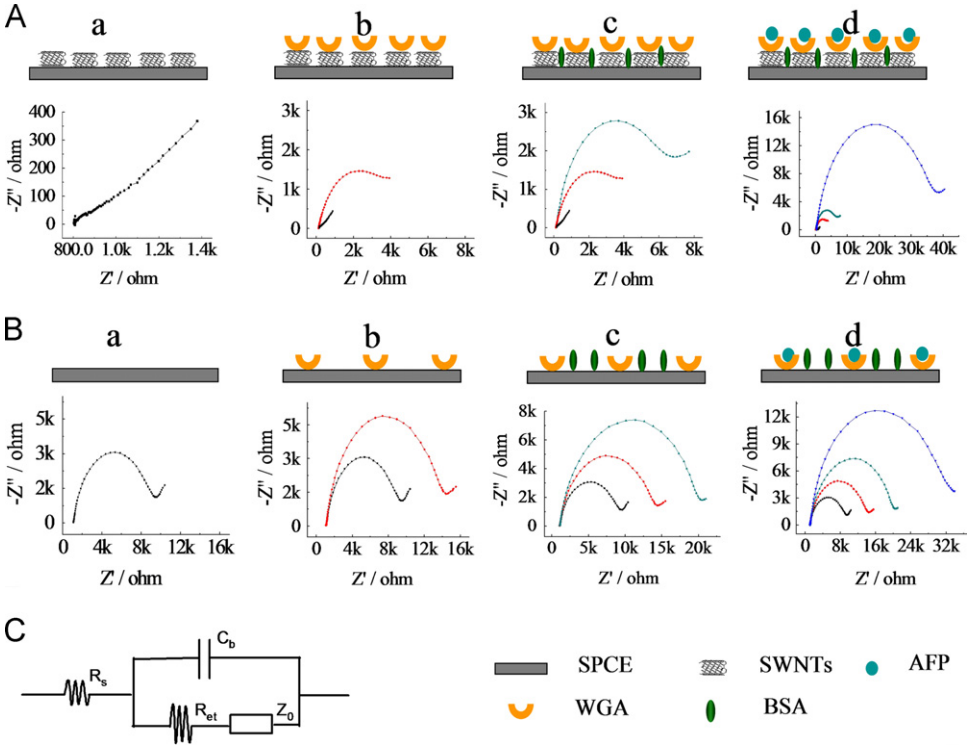


Fig. 1. The Nyquist plots of impedance spectra obtained at (A) WGA/SWNTs/SPCE and (B) WGA/SPCE. A: (a) SWNTs/SPCE, (b) WGA/SWNTs/SPCE; (c) BSA blocked WGA/SWNTs/SPCE; and (d) the WGA-based biosensor incubated with 1 ng/L AFP for 60 min. B: (a) SPCE, (b) WGA/SPCE, (c) BSA blocked WGA/SPCE, and (d) the biosensor incubated with 1 ng/L AFP for 60 min. The EIS measurements were carried out in 10 mM PBS (pH 7.4) containing 0.10 M KCl and 5 mM $K_3[Fe(CN)_6]$/5 mM $K_4[Fe(CN)_6]$, applying amplitude 5 mV in the frequency range of 0.1–100 kHz. (C) Equivalent circuit. Fitted data (solid line). Experimental data (dotted line).

the R_{et} measured 9.06 kΩ for the bare SPCE (Fig. 1B,a), 14.83 kΩ for the WGA/SPCE (Fig. 1B,b), and 21.87 kΩ for WGA/SPCE blocked with BSA (Fig. 1B,c). After incubation with 1 ng/L AFP, the value of R_{et} measured 36.57 kΩ (Fig. 1B,d). ΔR_{et}=14.70 kΩ on WGA/SPCE was obtained while ΔR_{et}=36.42 kΩ was obtained on WGA/SWNTs/SPCE for 1 ng/L AFP. Moreover, approximately 4.5-fold increase in R_{et} was obtained for the WGA/SWNTs/SPCE, but only 0.67-fold increase for the WGA/SPCE. This is ascribed to the fact that modification of the SWNTs layer onto the SPCE surface significantly decreases background and facilitates interfacial electron transfer, which is advantageous for sensitive detection of AFP. Additionally, a SWNTs network provides a high surface area and then increases both the density of immobilized WGA compared to the WGA/SPCE without SWNTs [26]. These results confirm that the WGA-based biosensor by employing SWNTs as amplification platform can be used to sensitively detect AFP.

AFM as a useful tool for surface imaging was employed to measure uniformity and thickness of the WGA/SWNTs surface. Fig. 2 shows the AFM images of a SWNTs-modified mica surface before and after immobilization of WGA. The observed surface roughness in the AFM image (Fig. 2A) is attributed to the presence of SWNTs bundles on the surface [30]. After immobilization of WGA to the SWNTs-modified mica surface, the roughness of the surface changed (Fig. 2B). The average *Z*-dimension values increase from ~88 nm to ~95 nm. This ~7 nm increase in height of the WGA-bound surface is consistent with the physical size of WGA as obtained by X-ray crystallography and AFM (~5.2–7.1 nm) [31,32]. Therefore, it is evident that immobilization of WGA on carboxyl SWNTs via a covalent approach was successful.

3.2. Optimization of WGA concentration and incubation time

Experimental parameters including the concentration of WGA during fabrication and the incubation time were optimized. As expected, at a fixed immobilization time, the concentration of WGA directly influences the surface coverage of the recognition element on the SWNTs/SPCE and further affects the sensitivity of the biosensor. Therefore, the concentration of WGA during fabrication was optimized. Fig. 3A shows the dependence of the R_{et} value on the concentration of WGA. Before incubation with AFP, the R_{et} values increase as the concentrations of WGA increase from 0.25 to 1.0 g/L, and then reach a plateau with concentrations from 1 to 2.5 g/L (Fig. 3A, curve a). After incubation with AFP, the R_{et} values of the biosensor show a trend similar to the values before the incubation (Fig. 3A, curve b). Fig.3A curve c shows that the ΔR_{et} value increases as the concentration of WGA increases from 0.25 to 1.0 g/L, likely as a result of an increase in the number of binding sites on the surface of the biosensor due to the increase in the surface coverage by WGA. With a further increase in the WGA concentration, the ΔR_{et} value reaches a plateau at about 1.0 g/L. The plateau is attributed to saturation of the surface coverage of WGA on the SWNTs/SPCE. Therefore, to minimize consumption of WGA, 1.0 g/L WGA was used in the following experiments for the fabrication of the biosensor.

Fig. 3B shows the influence of incubation time on the ΔR_{et} values when the biosensors are incubated with 1 ng/L AFP. The ΔR_{et} values increase sharply as the incubation time increases from 5 to 40 min and reach a plateau at 60 min. This result compares favorably to the incubation time of 60 min between

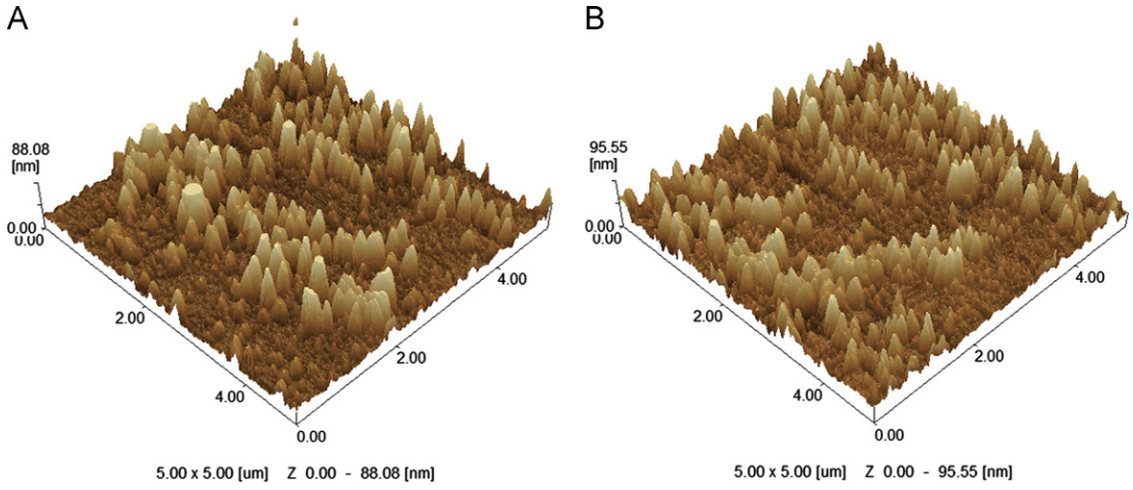


Fig. 2. AFM images of (A) SWNTs absorbed on mica substrate and (B) WGA covalently immobilized on SWNTs/mica.

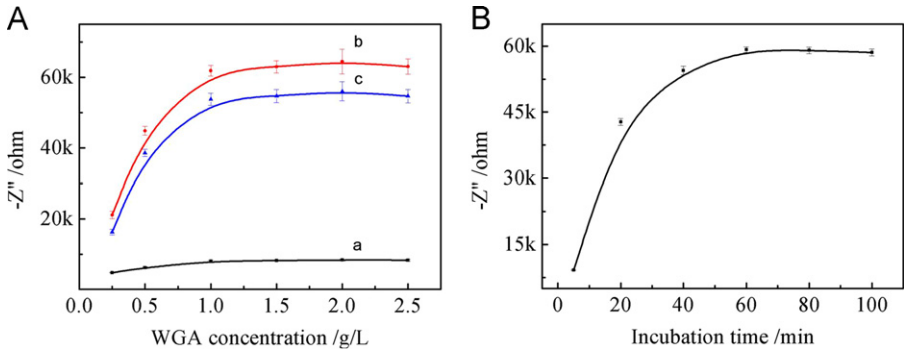


Fig. 3. (A) Dependence of R_{et} on the concentration of WGA before (a) and after (b) reacted with 1 ng/L AFP for 60 min, dependence of the increase of R_{et} on the concentration of WGA (c). (B) Dependence of R_{et} on incubation time for 1 ng/L AFP. EIS measurement condition was the same as in Fig. 1.

WGA immobilized on SPCE and the carbohydrates of cancer cells for an EIS biosensor [24,25]. This finding suggests that binding proceeded quickly and completed in ~60 min. To ensure efficient binding while using a short incubation time, an incubation time of 60 min was chosen for the following experiments.

3.3. Performance of the biosensor for AFP

The quantitative behavior of the WGA-based biosensor was assessed by measuring the dependence of ΔR_{et} on the concentration of AFP. Fig. 4 shows the Nyquist plots of the faradic impedance spectra for the WGA-based biosensor with different concentrations of AFP (0–100 ng/L). The inset of Fig. 4 shows the logarithmic relationship between ΔR_{et} and the concentration of AFP in the range of 1.0–100 ng/L. The regression equation was $\Delta R_{et}=11{,}957 \lg C+148{,}286$ (C is in units of ng/L) with a regression coefficient of 0.9890. The detection limit was 0.1 ng/L AFP (S/N=3). This detection limit is lower than those of previously reported biosensors such as the electrochemical immunosensor based on graphene sheet and multi-enzyme functionalized carbon nanospheres (10 ng/L) [11] and the electrochemical immunosensor based on an electrochemically addressing method for the fabrication of an immunosensor (50 ng/L) [8] (as shown in Table 1). However, the detection limit is slightly higher than the 0.5 ng/L reported for an electrochemical immunoassay based on target-induced release of biomolecules from magnetic carbon nanotubes [12].

The reproducibility of the WGA-based biosensor was estimated for the detection of 10 ng/L AFP. The relative standard deviation obtained was 4.6% using five individual biosensors and was 3.3% for seven independent measurements using the same biosensor. This indicates that the reproducibility of the fabricated biosensors is feasible. The storage stability of the EIS 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 96.5% of initial EIS value for 1 ng/L AFP.

The selectivity of the biosensor was assessed by testing AFP and non-glycosylated protein (BSA, HAS). A significant increase in R_{et} induced by the interaction of the WGA with 10 ng/L AFP ($\Delta R_{et}=62.73$ kΩ) was observed. On the other hand, very slight increase in R_{et} were found after the WGA-based biosensor was reacted with the other analytes, and they were $\Delta R_{et}=0.35$ kΩ (10 ng/L BSA), $\Delta R_{et}=0.42$ kΩ (10 ng/L HSA). Specifically, the measured value for AFP was close to the values for the mixtures of 10 ng/L AFP-10 ng/L BSA (62.91 kΩ) and for 10 ng/L AFP-10 ng/L HAS (62.67 kΩ). This indicates that the developed strategy has good selectivity for AFP.

The evaluation of the selectivity of the WGA-based biosensor was further performed by examining the R_{et} after mixing with three carbohydrates including a specific-binding carbohydrate (GlcNAc) [29] and nonspecific carbohydrates (glucose and rhamnose), according to the protocol described in electrochemical measurement. The measured ΔR_{et} values were 2.0 kΩ for GlcNAc, and only 0.15 kΩ for glucose and 0.12 kΩ for rhamnose. These results indicate that the fabricated WGA-based biosensor also can response to GlcNAc, which is attributed to the fact that WGA contains eight putative carbohydrate-binding sites for GlcNAc based on the principle binding domain of the aromatic residues stacking with the sugar ring, as well as the helper domain through hydrogen bonding [33,34]. The binding constant (K_b) of the immobilized WGA and GlcNAc was also determined using the proposed EIS method [35] to further understand the lectin–carbohydrate interactions. The K_b was calculated to be $2.99\times10^8\ M^{-1}$ from the plot of [GlcNAc]/ΔR_{et} as a function of [GlcNAc], representing a Langmuir isotherm adsorption (Fig. 5). This value is slightly higher than the $4.82\times10^7\ M^{-1}$ calculated by surface plasmon resonance SPR for WGA and monomeric GlcNAc

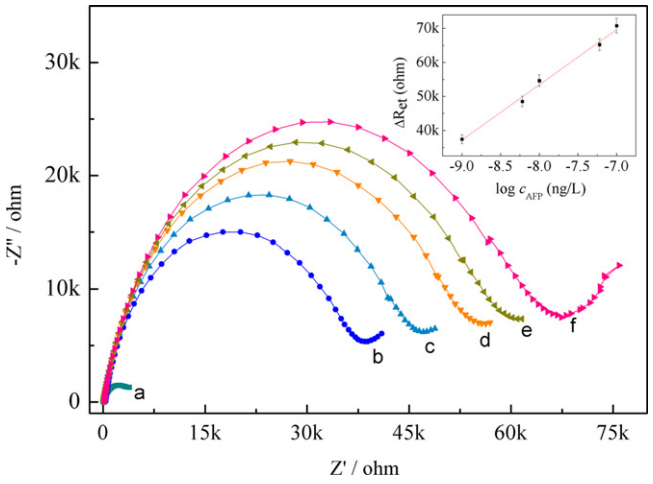


Fig. 4. The Nyquist plots of impedance spectra of the WGA-based biosensor for different concentrations of AFP: (a) 0 ng/L; (b) 1 ng/L; (c) 6 ng/L; (d) 10 ng/L; (e) 60 ng/L; and (f) 100 ng/L. Inset: dependence of ΔR_{et} on the logarithm of AFP concentration. EIS measurement condition was the same as in Fig. 1.

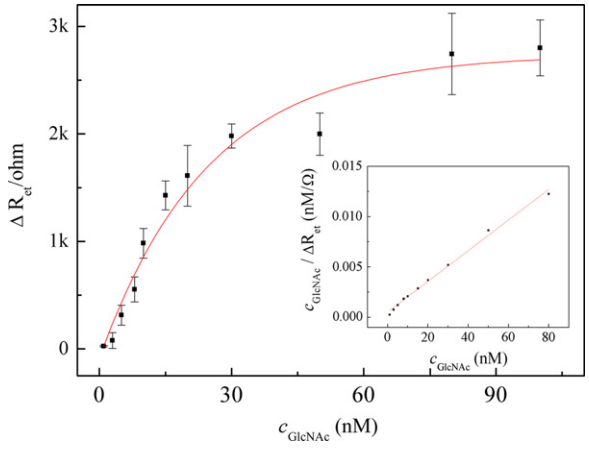
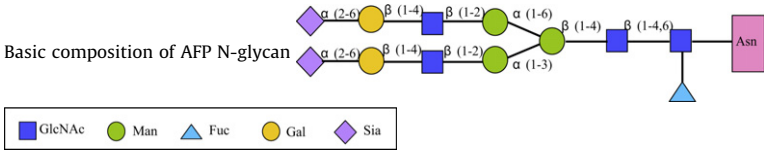


Fig. 5. Langmuir isotherms obtained from the WGA-based biosensor after incubation with different concentrations of GlcNAc for 60 min. Inset: linear regression between $c_{GlcNAc}/\Delta R_{et}$ and c_{GlcNAc}. EIS measurement condition was the same as in Fig. 1.

Table 1
Analytical performance for the analysis of AFP.

Detection technique	Recognition molecular	LOD (ng/L)	Linear range (ng/L)	Sample analysis	Refs.
Chronoamperometry	Antibody	50	100–50,000	Serum samples	[8]
Square wave voltammetry	Antibody	20	50–6000	Serum samples	[10]
Amperometry	Antibody	10	20–4000	Serum samples	[11]
EIS	Antibody	0.5	$1–2\times10^5$	Serum samples	[12]
EIS	Antibody	–	$2\times10^5–8\times10^5$	–	[13]
EIS	Lectin	0.1	1–100	Healthy and cancer patients serum samples	This work

Table 2
The chemical compositions of target glycoprotein sites to specifically bind with lectins and ΔR_{et} values obtained from five types of lectin-based biosensors after incubation with AFP and the serum samples from healthy individuals and from liver cancer patients.

Lectin	Specificity	AFP (kΩ)	Healthy individuals (kΩ)	Liver cancer individuals (kΩ)
WGA	(Neu5Ac[a]) GlcNAc[b] oligomers	42.23 ± 1.03	5.03 ± 0.13	4.56 ± 0.06
Con A	Branched and terminal Man[c] Man α-3[Manα-6] Man	23.86 ± 0.76	7.75 ± 0.42	3.42 ± 0.20
DSA	GlcNAc β-1,4GlcNAc oligomers	6.65 ± 0.11	4.21 ± 0.05	5.68 ± 0.30
LCA	Complex (Man/GlcNAc core with α-1,6 fucose)	1.31 ± 0.10	1.01 ± 0.02	2.10 ± 0.09
SNA	Siactose α-2,6Gal[d]/GalNAc[e]	0.96 ± 0.06	0.85 ± 0.04	4.65 ± 0.22
Basic composition of AFP N-glycan				

[a] Neu5Ac: N-acetylneuraminic acid.
[b] GlcNAc: N-acetylglucosamine.
[c] Man: mannose.
[d] Gal: galactose.
[e] GalNAc: N-acetylgalactosamine. (ΔR_{et} ± SD, n=5).

[36]. This large K_b value suggests that the binding strength between GlcNAc and surface-confined WGA is significantly strong.

3.4. Analysis of glycan expression on AFP N-glycan

To evaluate the glycan expression of AFP N-glycan and discriminate AFP between healthy and cancer patients serum samples, a lectin-based biosensors array was fabricated by individually immobilizing five lectins, including DSA, Con A, WGA, LCA and SNA [37], on five SWNTs/SPCE. The sequence of ΔR_{et} values (Table 2, third column) obtained for the lectin-based biosensors array upon incubation with 1 ng/L AFP is the WGA-based biosensor (42.23 kΩ) > Con A-based biosensor (23.86 kΩ) > DSA-based biosensor (6.65 kΩ) > LCA-based biosensor (1.31 kΩ) > SNA-based biosensor (0.96 kΩ). This sequence is in agreement with the bioaffinity that is expected based on AFP N-glycan possessing a N-linked glycan at asparagine 232, which contains mannose, GlcNAc, sialic acid and galactose, in biantennary or triantennary structures with a bisecting GlcNAc [38,39], as shown in the insert scheme of Table 2. The observed binding trend is same as that of the lectin-binding specificities reported in literature [38]. Thus, the proposed strategy could preliminarily evaluate the glycan expression of AFP using the fabricated lectin-based biosensors array.

To test the performance of the designed biosensors array in serum samples, the glycan expression patterns of serum AFP from both healthy and liver cancer patients were analyzed using the five lectin-based biosensors array described above. The serum samples were a gift from the Shaanxi Cancer Hospital. The serum samples were diluted by the binding buffer to an appropriate concentration, generally 1:100 (V/V) serum:buffer. The EIS measurement was performed as described in section 2.3. Table 2 lists the ΔR_{et} values obtained from the five different lectin-based biosensors array after incubation with the serum-diluted samples from healthy individuals (fourth column) and from liver cancer patients (last column). Comparing these values shows that the ΔR_{et} obtained from healthy serum samples are higher than those from liver cancer serum samples using the WGA- and Con A-based biosensors, whereas the ΔR_{et} obtained from healthy serum samples are lower than those from liver cancer serum samples using the DSA-, LCA- and SNA-based biosensors. It has been reported that strong bioaffinity occurs in DSA with more than two antennaries and aberrant bi-antennary of AFP N-glycan, in LCA with core fucose of AFP N-glycan and in SNA with α2–6-linked sialic acid of AFP N-glycan [39,40]. According to this logic, it is reasonable to deduce that the AFP from liver cancer serum has more core fucosylation, antennaries, and α2-6 sialylation than AFP from healthy serum. This speculation is consistent with literature reports that the glycan expression of AFP from liver cancer serum is different from expression from benign diseases [38,39]. Therefore, the biosensors in this work with different lectins as biological recognition molecules are potentially useful for the clinical diagnosis of early stage cancer [40].

4. Conclusion

A label-free EIS biosensor array for the detection of AFP has been developed by employing WGA as recognition element with high sensitivity, satisfactory reproducibility and good selectivity. SWNTs as immobilization platform for the WGA/SWNTs/SPCE biosensor reduce the background signal and enhance the EIS response. Letin-based biosensors array fabricated with different lectins can be used to evaluate the glycan expression of AFP N-glycan and can be applied to discriminate AFP between healthy and cancer patients serum samples. The strategy proposed in this work could further be used for high-throughput, label-free profiling of the glycan expression of cancer-related glycoproteins and to develop method and biosensor for cancer diagnosis in the early stages.

Acknowledgments

Financial supports from the National Science Foundation of China (No. 21027007, 20975065 and 20805028), the Fundamental Research Funds for the Central Universities (No. GK201002027) and the Doctoral Science Foundation of Yuncheng University (No. YKU2012018) are gratefully acknowledged. We thank Yuan Nan in the Shaanxi Cancer Hospital for providing the human blood samples.

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