Electrochemical lectin-based biosensor array for detection and discrimination of carcinoembryonic antigen using dual amplification of gold nanoparticles and horseradish peroxidase

Lingzhi Zhao[#], Congcong Li[#], Honglan Qi^{*}, Qiang Gao, Chengxiao Zhang
Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry and Chemical Engineering, Shaanxi Normal University, Xi'an, 710062, P.R. China

[#] co-first author; ^{*} To whom correspondence should be addressed. E-mail: honglanqi@snnu.edu.cn (HL Qi); Phone: +86 29 81530726; Fax: +86-29-81530727.

Abstract

An electrochemical lectin-based biosensor array employing lectin as molecular recognition elements was developed for sensitive detection and discrimination of carcinoembryonic antigen (CEA) based on dual signal amplification of gold nanoparticles and enzymatic catalysis. The electrochemical biosensor was fabricated by self-assembly of cysteamine on the surface of gold nanoparticle-modified screen-printed carbon electrodes and subsequently covalently coupling lectins on the surface of the cysteamine-modified electrode via the amidation reaction. A lectin–target–antibody sandwich-type conjugate was formed when the biosensor was successively reacted with target protein and horseradish peroxidase labeled anti-CEA antibody probe. In the presence of hydroquinone and hydrogen peroxide, the resulted biosensor produced an obvious catalysis current signal. The catalytic current at -0.25 V was linear with the concentration of CEA in the range of 1 ng/mL to 10 ng/mL with detection limit of 0.03 ng/mL and 0.05 ng/mL using concanavalin A and wheat-germ agglutinin as molecular recognition element, respectively, and in the range of 0.5 ng/mL to 7 ng/mL with detection limit of 0.01 ng/mL using *Lens culinaris* agglutinin as molecular recognition element. Additionally, the lectin-based biosensor array was successfully applied to evaluate the glycan expression of CEA N-glycan and discriminate CEA between healthy and cancer serum samples. The lectin-based biosensor array endows a feasibility tool for clinical diagnosis in complex biological systems and shows great promise for cancer detection and further pathological study of carcinogenesis.

Keywords: *Electrochemical biosensor, Array, Lectin, Carcinoembryonic antigen, Amplification*

1. Introduction

Cancer has threatened human health seriously and it has been frequently reported that there are the significant correlation between cancer and glycan alteration of the glycoproteins in oncogenic transformation, cell differentiation, and metastasis [1, 2]. Alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) as majority of the tumor markers, are both glycoproteins which have glycosylation sites in their amino acid sequences and have specific cancer-associated carbohydrate alterations when compared with its normal counterpart [3-5]. By employing specific lectin or monoclonal antibodies, carbohydrate alterations of glycoproteins can be detected for pathological study of carcinogenesis. For example, we previously developed a label-free electrochemical impedance spectroscopy biosensor array based on lectin functionalized carbon nanotubes for the detection and discrimination of glycosylation of AFP between healthy and liver cancer patients serum samples [6]. CEA, with a molecular weight of 180 kDa, is a member of cell surface glycoproteins and exists in 60-70% of tumor cells. CEA as one of tumor markers in patients is associated with certain tumors including breast tumors, colon tumors, ovarian carcinoma and cervical carcinomas [6-10]. Thus, accurate detection of CEA in serum and tissue shows promising applications in clinical tests for early discovery and diagnosis of cancer.

Extensive efforts have been devoted to the determination of CEA based on the various methods such as thermal lens microscope [12], fluorescence immunoassay [13], chemiluminescence immunoassay [14], surface-enhanced Raman scattering immunoassay [15] and electrochemical immunoassay [16-26] and so forth. Among them, electrochemical immunosensors have recently gained increasing attention in the quantitative detection of CEA owing to low cost, fast response, and ease of handling and miniaturization. To improve the sensitivity of electrochemical immunosensors,

various signal amplification strategies have been developed. One of most popular strategies is using nanomaterials including carbon nanotube [16], graphene [17-20], and noble metal nanoparticles such as gold [21], silver [22], platinum [23] and their composite [24-26] as signals or carriers to load multiple enzymes to amplify the signal of immunosensors. In these reports, most of immunosensors for the detection of CEA were based on a sandwich-type electrochemical immunoassay with the anti-CEA antibody as capture antibody. However, the antibody drawbacks associated with its production, stability and manipulation have prompted researchers to seek alternatives [27]. Lectin is now explored as novel molecular recognition elements for biosensor applications in the analysis of their cognate carbohydrates and glycoproteins due to its high specificity and diversity [28]. Compared with antibody-based immunoassay, the lectin-based bioassay could detect not only the content but also the aberrant glycosylation of the tumor markers.

The aim of this work is to develop lectin-based biosensor array with different lectins as molecular recognition elements to detect the content and the aberrant glycosylation of CEA with high specificity and high-throughout. Here, for the first time, an electrochemical lectin-based biosensor array was developed for the detection and discrimination of CEA using dual amplification of gold nanoparticles and horseradish peroxidase. The principle scheme is demonstrated in Fig. 1. Gold nanoparticles (GNPs) were firstly electrodepositing onto a screen-printed carbon electrodes (SPCEs) to form the GNPs modified SPCEs (GPE). The electrochemical biosensor was fabricated by self-assembly of cysteamine on the surface of GPE and subsequently covalently coupling lectins on the surface of the cysteamine-modified electrode via the amidation reaction. After sandwich immunoassay of target CEA and horseradish peroxidase (HRP) labeled anti-CEA antibody, a lectin-CEA-antibody

sandwich-type conjugate formed onto the electrode surface. In the presence of hydroquinone and hydrogen peroxide, a catalysis current signal produced, which was directly proportional to the concentration of CEA. Moreover, a lectin-based biosensor array was fabricated using different lectins, including concanavalin A, wheat-germ agglutinin and *Lens culinaris* agglutinin, and was applied to preliminarily evaluate the glycan expression of CEA N-glycan and to discriminate CEA between of healthy and cancer serum samples. Thus, the proposed method shows great promise for the early clinical diagnosis of malignant tumour and further pathological study of carcinogenesis.

2. Experimental

2.1 Reagents and Apparatus

Lectins including wheat-germ agglutinin (WGA) from *Triticum vulgare* (wheat), *Lens culinaris* agglutinin (LCA), concanavalin A (Con A) type IV from *Canvalia ensiformis* seeds, C-reactive Protein (CRP), H₂AuCl₄, cysteamine, N-(3-dimethylamino-propyl)-N'-ethylcarbo-dimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (USA). Prostate specific antigen (PSA) and carcinoembryonic antigen (CEA) were obtained from Fitzgerald Industries International, Inc. (USA). HRP labeled anti-CEA antibody and human immunoglobulin G (IgG) were obtained from Beijing middle mountain gold bridge biological technology Co., LTD. (China). Albumin chicken egg protein was obtained from Sino-American Biotechnology Co., Ltd. (China). Bovine serum albumin (BSA) was obtained from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (China). Hydroquinone (HQ) and horseradish peroxidase (H₂O₂) were from Xi'an Third Chemical Reagent Company (China).

Lectin (except Con A), CEA and other protein solutions were prepared in 10 mM

phosphate buffer saline (PBS, pH 7.4, 10 mM $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ and 100 mM KCl). Con A was prepared in 10 mM PBS containing 1 mM CaCl_2 and 1 mM MnCl_2 . 10 mM PBS was used as washing buffer. All chemical reagents were of analytical grade and used as received, and aqueous solutions were prepared with Milli-Q water. Clinical serum samples were obtained from Shaanxi Cancer Hospital.

Electrodag 423SS (carbon ink) and Electrodag 452SS (insulating dielectric oil) were purchased from Acheson Henkel Corporation (USA). BY-2100 silver paste was obtained from Shanghai Bao Yin Electronic Materials Ltd. (China). An epoxy film (0.5 mm thickness) was used as the base substrate of printed electrode.

Cyclic voltammetric and amperometric measurements were carried out with a CHI 1030 multi-channel electrochemical workstation (Shanghai Chenhua Instrument Co. Ltd., China). Electrochemical impedance spectroscopy (EIS) was performed on a CHI 660 electrochemical workstation (Shanghai Chenhua Instrument Co. Ltd., China). Scanning electron microscopy (SEM) images elemental analysis (EDS) were acquired from a Quanta 200 (FEI Co. USA).

2.2 Fabrication of GNPs modified SPCEs and preparation of the biosensor

A base electrode array consisting of eight working SPCEs, one auxiliary electrode and one Ag/AgCl reference electrode were fabricated by screen-printing technology according to the references [29, 30]. The procedure for the deposition of GNPs on SPCEs was adapted from the reference [31]. Briefly, GNPs were electrodeposited on the electrode array by holding a constant potential of + 1.1V (vs. Ag/AgCl, sat. KCl) for 60 s and then a constant potential of -0.1 V for 10 min at desired electrode in 0.1 M HClO_4 containing 0.1% HAuCl_4 .

The biosensor was fabricated as following three steps. Firstly, 500 μL of 20 mM cysteamine solution was dropped onto the surface of freshly prepared GNPs modified

SPCEs and incubated overnight at room temperature in dark. Then, 20 μ L of 2 mg/mL different lectins (Con A, WGA or LCA) activated by 20 mg/mL EDC and 20 mg/mL NHS was dropped onto the desired electrode for 2 hour at room temperature. Finally, the electrodes were subsequently blocked with 1% BSA for 40 min. After each step, the electrode was rinsed thoroughly with washing buffer to remove adsorption components. The lectin-based biosensor array fabricated with different lectins, including WGA, LCA and Con A, were prepared by immobilizing each lectin onto different GNPs modified SPCE surface, respectively.

As a control, a biosensor fabricated by physical adsorption was obtained by dropping 20 μ L of 2 mg/mL Con A onto the desired gold nanoparticles modified SPCEs surface for 2 h, and then blocked with 1% BSA for 40 min.

2.3 Electrochemical measurements

20 μ L of different concentrations of CEA or serum samples were dropped onto the fabricated biosensor or biosensor array for 60 min and then rinsed with washing buffer. The fabricated biosensor was then immersed in 100 μ L of 1:1000 HRP-labeled anti-CEA antibodies for 40 min and then rinsed with washing buffer. For electrochemical measurement, the resulted biosensor was immersed into 1.0 mL of 0.10 M PBS (pH 7.4, 0.10 M $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ and 0.1 M KCl) containing 1.5 mM HQ and 2.0 mM H_2O_2 . The chronoamperometry measurements were performed at an applied potential of -0.25 V (vs. Ag/AgCl, sat. KCl). The concentration of CEA was quantified by the increased current response ($\Delta\text{current}=I_s-I_0$), where I_s and I_0 are the steady-state current (obtained from 8 s~10 s) in the presence and absence of CEA, respectively. All electrochemical measurements were carried out at room temperature.

3. Results and discussion

3.1 Fabrication and characterisation of lectin-based biosensor

Here, an electrochemical lectin-based biosensor array was developed for the detection and discrimination of CEA using dual amplification of gold nanoparticles and horseradish peroxidase. Firstly, GNPs were selectively electrodeposited onto desired SPCEs for the immobilization of lectin. In order to validate the in-situ forming of the GNPs by electro-deposition on the SPCEs, scanning electron microscopy (SEM) and elemental analysis (EDS) were used to characterize the morphology and composition of the GNPs, respectively (as shown in Fig. 2). In details, after the electrodeposition of GNPs on the SPCEs for 10 min, SEM images depict that the surface of SPCEs was modified with homogeneous spherical nanoparticles (Fig. 2a, b) and EDS spectrogram of two kinds of interfaces indicate that these white spherical dots were GNPs (Fig. 2c, d). The results indicate that GNPs have successfully been electrodeposited on the SPCEs.

GNPs have two functions in this work. One is amplification of the active surface area and the other is providing sites for self-assembly of cysteamine. In order to illustrate the function of GNPs, active surface area of electrodes and the feasibility of fabrication of the electrochemical biosensor were examined. The SPCEs and GNPs modified SPCEs were electrochemically characterized by cyclic voltammogram (CV) in 5.0 mM $K_3[(FeCN)_6]$ containing 0.1 M KCl (seen Fig. S-1 A). CV curves of SPCEs were well-overlapped and had a total active surface of 0.011 cm² based on the Randles–Sevcik equation [32]. An obvious increase of current and a couple of sharper redox peaks were obtained at GNPs modified SPCEs (seen Fig. S-1B). The active surface area increased to 0.042 cm² for GNPs modified SPCE. GNPs increase the active surface area by 3.8 times compared with that of SPCEs.

The lectin-based biosensor was fabricated by self-assembly of cysteamine on GNPs modified SPCEs surface, followed by covalently coupling lectin onto the

surface of the cysteamine-modified electrode via the amidation reaction. Con A was used as model to show the feasibility of fabrication process. The different interfaces of modified electrodes were evaluated by monitoring the change of electron-transfer resistance (R_{et}) in EIS and conductivity in CV with $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as the redox probe (seen Fig. S-2). The EIS results in Fig. S-2A show that the impedance spectra contain a semicircular portion at higher frequencies and a linear portion at lower frequencies, which corresponds to the R_{et} and the diffusion process, respectively. R_{et} (Fig. S-2A, b, $R_{et}=2.53 \text{ K}\Omega$) at GNPs modified SPCE was much smaller than that at a bare SPCE (Fig. S-2A, a, $R_{et}=14.29 \text{ K}\Omega$), indicating that GNPs can accelerate the electron transfer owing to its excellent electronic conductivity. The R_{et} at cysteamine modified electrode greatly decreased to $0.73 \text{ K}\Omega$ (Fig. S-2A, c), which is mainly attributed to the fact that cysteamine is positively charged in solution at pH 7.4, which is beneficial to the diffusion of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ to electrode surface. The R_{et} at Con A (pI = 5) modified electrode greatly increased to $1.24 \text{ K}\Omega$ (Fig. S-2A, d) and the R_{et} further increased to $3.53 \text{ K}\Omega$ after blocking with BSA (pI = 4.8-5.0) (Fig. S-2A, e). After 5 ng/mL CEA incubating with Con A, the R_{et} continued to increase to $5.46 \text{ K}\Omega$ (Fig. S-2A, f). These increases are mainly ascribed to the insulating properties of the proteins Con A, BSA and CEA, which hinders the access of the redox probe to the electrode surface from the solution. After HRP-labeled anti-CEA antibody was incubated with the CEA immobilized electrode, the binding of anti-CEA antibody to CEA through specific recognition further led to higher R_{et} value, which markedly increased to $7.82 \text{ K}\Omega$ (Fig. S-2A, g). The above results of Nyquist plots are also in accordance with CV data perfectly (seen Fig. S-2B), suggesting that GNPs, cysteamine, Con A, BSA, CEA and anti-CEA antibody have been successively assembled onto SPCEs in sequence and the as-designed electrochemical biosensor has been

successfully fabricated.

3.2 Feasibility of the lectin-based biosensor

Con A was firstly taken as one of molecular recognition element to study the feasibility of the lectin-based biosensor in assay of CEA. The electrochemical detection is based on the catalysis of the immobilized HRP toward the reduction of H_2O_2 with hydroquinone as mediator. Fig. 3A displays chronoamperometric profiles of the lectin-based biosensor for the detection of different concentration of CEA in 0.1 M PBS (pH 7.4) containing 1.5 mM HQ and 2.0 mM H_2O_2 at -0.25 V. It can be observed that the absolute value of the steady-state reduction current of 10 ng/mL CEA (Fig. 3A, line d, 0.68 μA) is much bigger than that of the blank (Fig. 3A, line a, 0.21 μA), 1.0 ng/mL CEA (Fig. 3A, line b, 0.36 μA) and 5.0 ng/mL CEA (Fig. 3A, line c, 0.52 μA).

A biosensor fabricated by directly physical adsorption of Con A onto GNPs modified SPCEs was used as control to assay of CEA in order to show the function of cysteamine. The results are shown in Fig. 3B, it can be seen that the absolute value of the steady-state reduction current was 0.40 μA , 0.28 μA , 0.23 μA , 0.17 μA for 10 ng/mL CEA, 5.0 ng/mL CEA, 1.0 ng/mL CEA, and 0 ng/mL CEA, respectively, which are much smaller than those obtained by the biosensor fabricated by covalent method. These results evidently support that the electrochemical detection of CEA using the as-fabricated Con A-based biosensor is feasible and preponderant.

3.3 Optimization of the experimental parameters of the lectin-based biosensor

To obtain excellent analytical performance, experimental parameters including the applied potential and the incubation time were optimized. Fig. S-3A shows effects of applied potential on the current of the biosensor for 1.0 ng/mL CEA. It can be seen that the increased current response sharply increase with negatively shifting potential

from -0.05 to -0.20 V and then reach the maximum at -0.25 V and slightly reduce with further negatively shifting potential. Therefore, a constant potential of -0.25 V (vs. Ag/AgCl) was selected as the working potential for the following experiments. Fig. S-3B shows the influence of incubation time on the current when the biosensors are incubated with 1.0 ng/mL CEA. The current increased greatly with increase of incubation time from 15 to 45 min, and trended to the constant values after the incubation time of 60 min, indicating binding completed in 60 min. Thus, we employed the incubation time of 60 min for the following experiments.

3.4 Analytical performance of the lectin-based biosensor array

Under the optimized conditions, the quantitative behaviour of the biosensor array was examined by measuring the dependence of the current on the concentration of CEA. Three kinds of lectins, including Con A, WGA and LCA, as molecular recognition elements were employed to fabricate the lectin-based biosensor array. The results showed that the current response increases with the increase of the concentration of CEA with three lectins separately. The increased current response was directly proportional to the concentration of CEA in the range from 1.0 ng/mL to 10 ng/mL using Con A and WGA as molecular recognition elements (as shown in Fig. 4A, B). The regression equation were $\Delta\text{current}_1 (\mu\text{A}) = 0.034C + 0.14$, $R_1 = 0.9855$ and $\Delta\text{current}_2 (\mu\text{A}) = 0.026C + 0.19$, $R_2 = 0.9869$ (unit of C is ng/mL). The detection limit of CEA is calculated to be 0.03 ng/mL and 0.05 ng/mL (3σ) [33], respectively. Specifically, with LCA as molecular recognition element, the increased current response was directly proportional to the concentration of CEA in the range from 0.5 ng/mL to 7 ng/mL (as shown in Fig. 4C). The regression equation was $\Delta\text{current}_3 (\mu\text{A}) = 0.094C + 0.41$, $R_3 = 0.9950$, and the detection limit of CEA was 0.01 ng/mL. Compared with other CEA immunosensors, the biosensor displayed good

performance in the detection limit and the linear range (Table 1) [34-43]. For example, the detection limit is lower than those of biosensors such as the electrochemical immunosensor based on SiO₂/Thionine nanocomposite (0.34 ng/mL) [36] and chitosan–carbon nanotubes–gold nanoparticles nanocomposite (0.04 ng/mL) [42] (Table 1). These results sufficiently indicate that the employment of gold nanoparticles and horseradish peroxidase catalysis for dual signal amplification are successful.

The binding constant of the biosensor was calculated to evaluate the specific binding ability between lectins and glycan. Assume that all kinds of lectins interacted with glycan were in accord with the langmuir isotherm, and the combination of each binding site had the same ability. In this work, the combination ability is related with the increased current response of the biosensors after incubating with CEA, and the coverage and current relationship complies with the following equation (1). Among them, Current (0) (I_0) and Current (I) (I_s) are current value of the biosensor before and after incubating with CEA, respectively.

$$\theta = 1 - [\text{Current}(0) / \text{Current}(I)] \quad (1)$$

In the Langmuir isotherm, the binding constant K_a can be expressed by the following equation (2). Among them, K_a is the binding constant and C is concentration of analyte. Combining equation (1) and (2), available relation can be obtained as shown in equation (3).

$$\theta = K_a C / (1 + K_a C) \quad (2)$$

$$K_a C = [\text{Current}(I) - \text{Current}(0)] / \text{Current}(0) \quad (3)$$

Taking the logarithm on both sides of equation (3), equation (4) can be obtained

by deformation and consolidating. It can be concluded that in certain concentration range, the $\lg(\Delta \text{Current} / \text{Current}(0))$ is directly proportional to $\lg C$, and the vertical intercept is $\lg K_a$, from which binding constant K_a can be calculated, as shown in Fig. 5, the calculation results are shown in Table 2. Other related research has proved that the binding constants of glycan and lectin can be variant due to the different measuring method and fixed quantity [44].

$$\lg[\Delta \text{Current} / \text{Current}(0)] = \lg K_a + \lg C \quad (4)$$

The selectivity of the biosensor was assessed by testing the specific binding protein (1.0 ng/mL CEA) and nonspecific proteins (100 ng/mL other proteins, including BSA, CRP, IgG, egg protein, PSA). A distinct increase in current ($\Delta \text{Current} = 0.148 \mu\text{A}$) was observed for 1.0 ng/mL CEA when quite slight increase in current were found for the other protein (seen Fig. S-4). A distinct increase for CEA was ascribed to the high binding affinity of Con A towards CEA. These results indicate that the proposed strategy revealed sufficiently selective for the detection of CEA.

3.5 Analysis of glycan expression on CEA N-glycan

The developed method was further implemented in the analysis of clinical serum samples from healthy and liver cancer patients to illustrate the feasibility of the electrochemical lectin-based biosensor array for clinical applications. Serum samples were obtained from Shaanxi Cancer Hospital according to the rules of the local ethical committee. The results are listed in Fig. 6. Both of the increased current responses were obtained for healthy and cancer serum samples at Con A-, WGA- and LCA-based biosensor compared with blank and the increased current response at LCA-based biosensor is higher than that of Con A-, WGA- based biosensor, indicating that Con A, WGA and LCA all have their carbohydrate-binding specificity

for CEA and the N-glycan structure expressed high abundance of LCA-recognizing glycan and low abundance of Con A- and WGA-recognizing glycan. Compared with the results in healthy and cancer serum, it can be seen that the increased current response obtained from healthy serum samples are higher than those from liver cancer serum samples using Con A-based biosensor and WGA-based biosensor, whilst the increased current response obtained from healthy serum samples are lower than those from liver cancer serum samples using the LCA- based biosensor. The results indicate that the glycan structures of CEA from liver cancer are different from that healthy serum. It was previously reported that the structure of CEA N-glycan was mainly composed of core fucose, rectenna or antenna structure double branch N-acetyl glucosamine, galactose, mannose, etc [45, 46]. It is well-known that Con A bind with high affinity to the core trimannoside moiety [47], WGA bind with N-acetylglucosamine oligomers [48] and LCA bind with Man/GlcNAc core with α -1,6 fucose [49]. Thus, the content of core fucose in liver cancer serum samples is more than that in healthy serum samples, while the content of mannose and N-acetylglucosamine are less. These results are consistent with the previous report [50-52]. Hence, the biosensor array in this work with different lectins as molecular recognition molecules could be reasonably applied to determination of CEA levels in human serum and evaluation of the glycan expression of CEA N-glycan for the clinical diagnosis.

4. Conclusion

In this work, a simple and sensitive electrochemical biosensor array for the detection and discrimination of CEA have been developed employing three kind of lectins as molecular recognition elements and using gold nanoparticles as platform and horseradish peroxidase functionalized antibody as signal probe for dual signal

amplification. The fabricated biosensors show excellent analytical performance for CEA with low detection limit, good sensitivity, and wide linear range. Moreover, lectin-based biosensor array fabricated with different lectins can be used to evaluate the glycan expression of CEA N-glycan and discriminate CEA between healthy and cancer patients serum samples. These results indicate that the lectin-based bioassay by employing lectin–target–antibody reaction could be a promising alternative to the typical antibody–antigen–antibody sandwich immunoassay. The strategy proposed in this work indicates the lectin-based biosensor has good feasibility in clinical serum analysis and provides a reference for the early diagnosis of malignant tumor.

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Figures and captions

Fig. 1 Schematic representation of the electrochemical biosensor array for the detection of CEA

Fig. 2 SEM images of SPCE (a) and GNPs modified SPCE (b). EDS spectrogram of SPCE (c) and GNPs modified SPCE (d).

Fig.3 Chronoamperometric profiles of the biosensor based on covalently coupling method (A) and the biosensor based on physical absorption (B) for the detection of CEA. (A): (a) 0 ng/mL; (b) 1 ng/mL; (c) 5 ng/mL; (d) 10ng/mL. (B): (a) 0 ng/mL; (b) 1 ng/mL; (c) 5 ng/mL; (d) 10ng/mL. Detection conditions: Incubation time, 60 min; Applied potential, -0.25 V. The electrolyte solution: 0.1 mol/L PBS (pH 7.4) containing 1.5 mmol/L HQ and 2.0 mmol/L H_2O_2 .

Fig. 4 Chronoamperometric profiles of different lectins as molecular recognition elements for different concentration of CEA. Insert, the linear relationship between increased current response and the concentrations of CEA. (A) Con A as molecular recognition element, CEA concentration is (a) 0 ng/mL; (b) 1 ng/mL; (c) 3 ng/mL; (d) 5 ng/mL; (e) 7 ng/mL; (f) 10 ng/mL. (B) WGA as molecular recognition element, CEA concentration is (a) 0 ng/mL; (b) 1 ng/mL; (c) 3 ng/mL; (d) 5 ng/mL; (e) 7 ng/mL; (f) 10 ng/mL. (C) LCA as molecular recognition element, CEA concentration is (a) 0 ng/mL; (b) 0.5 ng/mL; (c) 1 ng/mL; (d) 3 ng/mL; (e) 5 ng/mL; (f) 7 ng/mL. The measurement conditions are same as Fig. 3.

Fig. 5 The relationship between $\Delta\text{Current}/\text{Current}(0)$ and the concentrations of CEA with (a) Con A, (b) WGA and (c) LCA. ($\Delta\text{Current} = \text{Current}(I) - \text{Current}(0)$)

Fig. 6 $\Delta\text{Current}$ values obtained on different lectin-immobilized electrodes after incubation with healthy and cancer serum samples, respectively. The measurement conditions are same as Fig. 3.

Table 1. Analytical performance for the analysis of CEA

Detection technique	Recognition molecular	LOD	Linear range	Sample analysis	Refs
Amperometric measurement	Antibody	0.3 ng/mL	0.5~ 120.0 ng/mL	serum samples	34
Amperometric measurement	Antibody	0.2 ng/mL	0.3~10 ng/mL	serum samples	35
Amperometric measurement	Antibody	0.34 ng/mL	1.0 ~ 100.0 ng/mL	serum samples	36
EIS	Antibody	0.1 pg/mL	1pg/mL~ 100 ng/mL	serum samples	37
Square wave voltammetry	Antibody	0.0175 ng/mL	0.025~ 250 ng/mL	serum samples	38
Differential pulse voltammetry	Antibody	0.01 ng/mL	0.01~100 ng/mL	serum samples	39
Differential pulse voltammetry	Antibody	4.6 pg/mL	0.01 ~50 ng/mL	serum samples	21
Differential pulse voltammetry	Antibody	0.013 ng/mL	0.05 ~20 ng/mL	serum samples	40
Cyclic voltammetry	Antibody	5 ng/mL	5~ 60 ng/mL	serum samples	41
Cyclic voltammetry	Antibody	0.04 ng/mL	0.1 ~ 2.0 ng/mL	serum samples	42
Electrochemical stripping voltammetry	Antibody	3.5 pg/mL	5.0 pg/mL~ 5.0 ng/mL	serum samples	43
Chronoamperometry	Lectin	0.01 ng/mL	0.5 ng/mL~ 10 ng/mL	serum samples	This work

Table 2 Association constants of different lectins with CEA

Lectin	Con A	WGA	LCA
$K_a /(\text{ng/mL})^{-1}$	0.44	0.48	0.60