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Detection of β-glucans using an amperometric biosensor based on high-affinity interaction between Dectin-1 and β-glucans

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ABSTRACT

Early diagnosis of fungal infection plays an important role in increasing antifungal therapeutic response, but meaningful tests such as microbiological cultures and histopathological diagnosis are usually insensitive and time-consuming. A sensitive amperometric biosensor for β-glucans was fabricated by immobilizing Dectin-1 onto Nafion–thionine–gold nanoparticle–chitosan multilayer films to trap its corresponding ligand from sample solution. On formation of ligand–receptor complex, detection of β-glucans was accomplished by monitoring the decrease of the electrochemical signal of the modified electrode due to the inhibition of the transmission of electrons. Dectin-1 was constructed by cloning the extracellular carbohydrate recognition domain of the mouse Dectin gene into the pET28a(+) prokaryotic expression vector. Optimal conditions and analytical performances of the described biosensor were investigated. Under the optimal conditions, the biosensor response for β-glucans presented good accuracy, stability, and reproducibility. The proposed biosensor not only could be used for rapid analysis of serum β-glucans but also provided a screening procedure for the determination of fungal infections.

Fungal infections have become increasingly prevalent during the recent decade along with the widespread use of antibacterial agents and the rapid increase of immune-compromised populations. Nowadays, the morbidity and mortality of fungal infection remain high [1–5]. Thus, early and accurate diagnosis can contribute to the timely treatment of patients and to the control of fungal infections. Currently, conventional methods such as microbiological culture and histopathological diagnosis are being used for determination of fungal infection. However, microbiological cultures are usually insensitive and time-consuming [6,7]. Moreover, histopathological diagnosis often requires invasive procedures to obtain the specimens [8]. Thus, there is an increasing need for rapid and accurate methods for determination of fungal infection. Recently, rapid diagnosis assays have been developed by determination of fungal cell wall components and fungal genomic DNA. Galactomannan can be detected by enzyme-linked immunosorbent assay (ELISA)[2] test based on monoclonal antibody to recognize the galactofuran epitopes of the galactomannan antigen released by fungi [9,10]. However, some antibiotics derived from penicillin can cause a cross reaction that can lead to false positive results in these ELISA-based assays [11,12]. Detection of fungal DNA theoretically should be a rapid and sensitive diagnostic method, but a small amount of free DNA in a sample would cause a lower positive detection rate, and the laborious sample pretreatment would also affect accuracy [13,14].

β-Glucans (BDGs) are conserved components of the cell wall of most medically important fungi, including *Candida*, *Aspergillus*, and *Fusarium*. BDGs can be detected spectrophotometrically by activating factor G, a coagulation factor of the horseshoe crab (kinetic turbidimetry). However, this method is time-consuming and complex to perform laborious sample pretreatment [15,16]. Thus, a rapid and specific method for BDG assay is essential in the determination of fungal infection. Dectin-1, a type II transmembrane receptor for BDGs, is widely expressed on phagocytes, which can specifically bind BDGs to trigger phagocytosis of fungi [17,18]. It was recently reported by Palma and coworkers that an oligosaccharide microarray can be used to investigate the interaction between Dectin-1 and BDGs [19]. The study indicated that the ligand–receptor complex can be formed between Dectin-1 and BDGs in vitro. Therefore, an assumption can be made that Dectin-1 can be identified as a molecular target recognizing BDGs, and this can provide a useful early screening procedure for the determination of fungal infection.

Recently, electrochemical biosensors have attracted a lot of attention because they can combine the high specificity of

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[2] *Abbreviations used:* ELISA, enzyme-linked immunosorbent assay; BDG, β-glucan; CRD, carbohydrate recognition domain; cDNA, complementary DNA; Thio, thionine; GNP, gold nanoparticle; CHIT, chitosan; BSA, bovine serum albumin; PCR, polymerase chain reaction; IPTG, isopropyl β-D-thiogalactopyranoside; EDTA, ethylenediaminetetraacetic acid; PEG, polyethylene glycol; NTA, nitrilotriacetic acid; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; FIFC, fluorescein isothiocyanate; IgG, immunoglobulin G; GCE, glassy carbon electrode; SCE, saturated calomel electrode; mRNA, messenger RNA; NCCLS, National Council of Clinical Laboratory Services.

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traditional immunoassay methods with the low detection limits and low cost of an electrochemical measurement system. So far, several different types of biosensors have been widely used in clinical diagnostics [20], biochemical analyses [21], food quality control [22], and environmental monitoring [23].

This article presents the construction of a Dectin-1-based sensitive biosensor for BDG detection. A Dectin-1 expression construct was generated by cloning the extracellular carbohydrate recognition domain (CRD) of the Dectin-1 gene from complementary DNA (cDNA) of mouse peritoneal macrophages into the pET28a(+) prokaryotic expression vector. The prototype biosensor was fabricated based on immobilization of Dectin-1 onto the Nafion–thionine–gold nanoparticle–chitosan (Nafion–Thio–GNP–CHIT) multilayer films to trap its corresponding ligand, BDGs. Analytical performances and optimal conditions of the described biosensor were investigated. Under the optimal conditions, the biosensor response for BDGs presented good accuracy, stability, and reproducibility. The biosensor was used to measure serum BDGs with satisfactory results.

Materials and methods

Reagents

pET28a(+), *Escherichia coli* DH5α, and *E. coli* BL21(DE3) were gifts from the College of Basic Medicine at Chongqing Medical University. Mice were purchased from Chongqing Medical University and were maintained in a specific pathogen-free environment in microisolator cages. Nafion, Thio, chloroauric acid, bovine serum albumin (BSA), CHIT, and BDGs were purchased from Sigma–Aldrich (Shanghai, China). CKT-5 M Set was purchased from Gold Mountainriver (Beijing, China). All other reagents were of analytical reagent grade and used without further purification. In addition, all of the solutions were prepared with ultrapure water from a Millipore Milli-Q system.

Apparatus

Cyclic voltammetric analysis was performed with a μAutolab III electrochemical workstation (Eco Chemie, Utrecht, The Netherlands). Kinetic turbidimetric assay was performed with an MB-80 Microbiology Kinetic Rapid Reader (Gold Mountainriver).

Preparation of Detecin-1

Total RNA was isolated from mouse peritoneal macrophages using the RNeasy Mini Kit (Qiagen, Shanghai, China) according to the manufacturer's instructions. cDNA was generated from 3 to 5 g of total RNA by using SuperScript II reverse transcriptase (Invitrogen, Chongqing, China) and random primers following the manufacturer's instructions. The cDNA encoding the extracellular CRD was amplified by polymerase chain reaction (PCR) with oligonucleotide primers P1 (5′-GCGCATATGCTAGCATTTTGGCGACAC-3′) and P2 (5′-AAACTCGAGCAGTTCCTTCTCACAGAT-3′) (where the underlined nucleotides in these oligonucleotides were *Nde*I and *Xho*I restriction sites, respectively). The PCR products were cloned into the pET28a(+) vector and had been verified by restriction endonuclease analysis. The recombinant was transformed into *E. coil* BL21(DE3) and was induced with isopropyl β-D-thiogalactopyranoside (IPTG) for 2 h at 37 °C, harvested, and sonicated. Inclusion bodies were dissolved in Bing buffer (pH 7.9) containing 8 M urea, 500 mM NaCl, 20 mM Tris–HCl, and 5 mM imidazole. The proteins were incubated with 5 mM reduced glutathione and 0.5 mM oxidized glutathione for 24 h at 5 °C and were successively dialyzed against decreasing concentrations of urea (6, 4, 2, and 1 M) in 0.01 M Tris–HCl (pH 7.9), 50 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), and 0.1% polyethylene glycol (PEG) 4000. Dialyzed proteins were purified by Ni^{2+}–NTA (nitrilotriacetic acid) affinity chromatography under the native conditions. Protein preparations were examined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE).

In vitro ligand–receptor binding experiments

The binding of Dectin-1 to *Candida albicans* was assessed by fluorescence microscopy and flow cytometry. The yeast form of *C. albicans* immobilized onto the slide was incubated with dialyzed with Dectin-1 at 25 °C for 1 h. The slide was washed with phosphate-buffered saline (PBS) and immersed in 10% horse serum at 25 °C for 1 h. After being washed three times with PBS, the resulting slide was incubated in mouse anti-His antibody solution at 25 °C for 1 h. The modified slide was washed with PBS and incubated in fluorescein isothiocyanate (FIFC)-labeled goat anti-mouse immunoglobulin G (IgG) at 25 °C for 1 h. Subsequently, the samples were observed under a fluorescence microscope.

Then 20 μl of the yeast form of *C. albicans* solution and 100 μl of Dectin-1 solution were added into an EP tube and incubated at 25 °C for 1 h. After that, 10% horse serum was added to the EP tube and incubated at 25 °C for 1 h. After being washed three times with PBS, mouse anti-His antibody solution was added to the EP tube and incubated at 25 °C for 1 h. The EP tube was washed with PBS and incubated in FIFC-labeled goat anti-mouse IgG at 25 °C for 1 h. Finally, the samples were analyzed with flow cytometry.

Preparation of GNP–CHIT

GNPs were prepared by adding sodium citrate solution to a boiling $HAuCl_4$ aqueous solution, as described previously [24]. CHIT powder (20 mg) was dissolved in 10 ml of 1.0% acetic acid solution and stirred at room temperature for 2 h until complete dissolution. Finally, CHIT solution was mixed with GNP solution by ultrasonic agitation to prepare the GNP–CHIT solution (1:1, v/v).

Preparation of biosensor

The biosensor was fabricated according to the following steps. First, the 3-mm-diameter glassy carbon electrode (GCE) was polished successively with 1.0, 0.3, and 0.05 μm alumina slurry. After rinsing thoroughly with ultrapure water, it was sonicated in absolute ethanol and ultrapure water for approximately 10 min and dried at room temperature. Then 5 μl of 1% (w/w) Nafion was dropped onto the electrode surface and allowed to dry in air at room temperature. In the second step, the pretreated electrode was scanned by cyclic voltammetry in the 0.1 mol/L PBS (pH 6.5) with 1 mmol/L Thio at room temperature. Third, the Nafion–Thio modified electrode was immersed in the GNP–CHIT solution for 2 h. Fourth, the Nafion–Thio–GNP–CHIT modified electrode was immersed in 1 ml of Dectin-1 solution at room temperature for approximately 12 h. Fifth, the resulting electrode was incubated in BSA solution for approximately 1 h at 35 °C to block possible remaining active sites and avoid nonspecific adsorption. Eventually, the multilayer film was formed on the electrode surface. The finished biosensor was kept dry in a refrigerator at 4 °C before use.

Electrochemical measurements of BDGs

Electrochemical measurements of BDGs were carried out in a conventional three-electrode cell composed of a saturated calomel electrode (SCE), a platinum wire counter electrode, and a modified working electrode. The analytical method was based on the variation of current response before and after ligand–receptor reaction,

which was evaluated as following the equation $\Delta i = i_1 - i_2$, where i_1 is the response current before the reaction and i_2 is the response current after the reaction.

Kinetic turbidimetric assay of BDGs

Kinetic turbidimetric analysis for BDGs was performed according to the manufacturer's instructions. Serum sample (0.2 ml) was put into the MB-80 Microbiology Kinetic Rapid Reader to react with the enzyme reagents. When the action ended, the results were calculated automatically.

Results and discussion

Expression of recombinant Dectin-1 CRD domain in E. coli

A cDNA fragment encoding the extracellular CRD of the mouse Dectin-1 gene was amplified from messenger RNA (mRNA) obtained from mouse peritoneal macrophages. As shown in Fig. 1A, a distinct DNA band of 552 bp was observed and show that the primers could produce the estimated sizes of PCR products of Dectin-1 under our experimental conditions. Then these PCR products were cloned into the pET28a(+) vector and verified by restriction digestion. Electrophoresis analysis indicated that the DNA band of approximately 552 bp was in agreement with the length prediction based on the nucleotide sequence of *AF262985* (the mouse Dectin-1 gene) (Fig. 1B). The recombinant vector pET28–CRD was transformed into *E. coli* BL21(DE3) and was induced with IPTG for 2 h at 37 °C, harvested, and sonicated. The protein was expressed and purified by Ni^{2+}–NTA affinity chromatography under the native conditions. As shown in Fig. 1C, the recombinant plasmids were expressed by induction with IPTG. Western blot analysis further revealed a 23-kDa product that is recognized by anti-His antibodies.

Binding of recombinant Dectin-1 CRD protein to C. albicans

The binding of Dectin-1 to *C. albicans* was examined by immunofluorescence staining and flow cytometry analysis. When *C. albicans* was incubated with Dectin-1, bright fluorescence was observed only on the slide (Fig. 2B). The fluorescence was localized around the cell wall portion of the yeast, indicating the binding of Dectin-1 protein to the outer cell wall of the yeast. In contrast, the yeast form of *C. albicans*, which was not incubated with dialyzed Dectin-1, served as a negative control (Fig. 2A). Likewise, the flow cytometric analysis confirmed these findings. The yeast form of *C. albicans* untreated with Dectin-1 served as the negative control

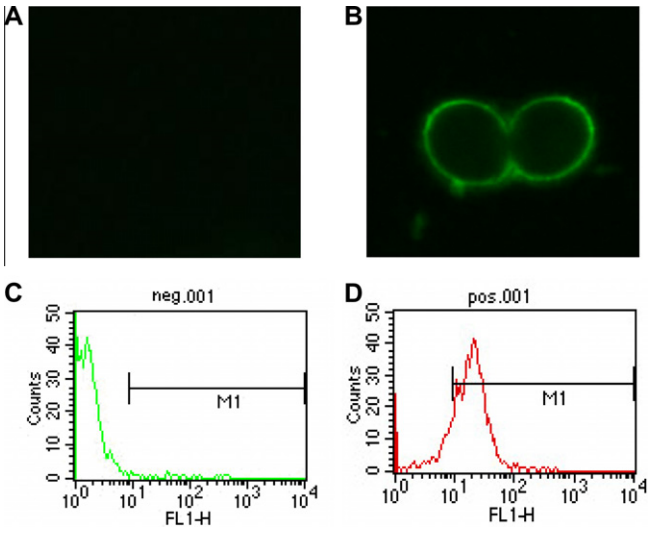

Fig. 2. (A and B) Immunofluorescence staining for *C. albicans* incubated without Dectin-1 (A) and with Dectin-1 (B). (C and D) Flow cytometric analysis for *C. albicans* treated without Dectin-1 (C) and with Dectin-1 (D). neg, negative; pos, positive.

(Fig. 2C). As shown in Fig. 2D, the fluorescence intensity of the yeast form of *C. albicans* treated with Dectin-1 was higher. This was likely due to the fact that the cell wall of *C. albicans* expresses high levels of Dectin-1-recognizing glycan. Our results demonstrating Dectin-1 and yeast glycan interaction are also in agreement with previously reported data [19]. Thus, the proposed strategy could be used to evaluate the binding of Dectin-1 to BDGs using ligand–receptor reaction-generated electrochemical signals.

Electrochemical behaviors of biosensor

Our strategy to construct a biosensor using Dectin-1 CRD is shown in Fig. 3. In this configuration, Dectin-1 is absorbed onto multilayer films, and the binding of glycan to Dectin-1 would affect the electric current on matrix and, thus, provide a measurement of the amount of binding between receptor and ligand. In a prototypical experiment, cyclic voltammograms of the differentially modified electrodes in different conditions were recorded and are shown in Fig. 4. No obvious peak current was observed in the Nafion modified electrode due to the lack of electron mediator (voltammogram a). However, in the voltammogram obtained for Nafion–Thio modified electrode (voltammogram b), the symmetri-

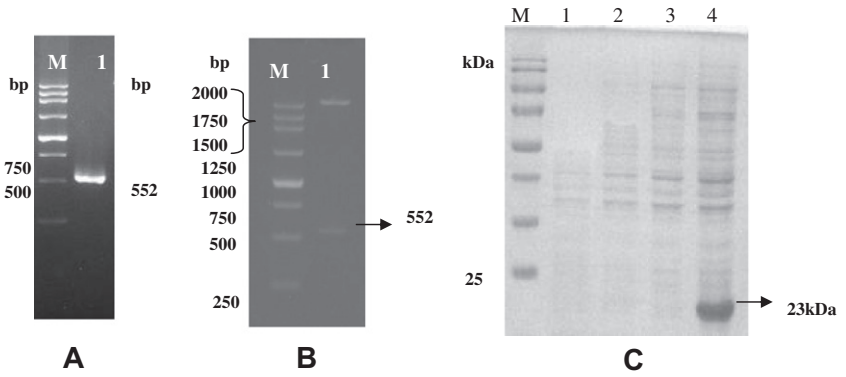

Fig. 1. Synthesis of Dectin-1. (A) PCR amplification of Dectin-1. Lane M: DNA marker; lane 1: PCR product of Dectin-1. (B) Identification of pET28–CRD. Lane M: DNA marker; lane 1: digestion of pET28–CRD with *NdeI* and *XhoI*. (C) Expression of pET28–CRD. Lane M: protein marker; lane 1: product of pET28 induced without IPTG; lane 2: product of pET28–CRD induced without IPTG; lane 3: product of pET28 induced with IPTG; lane 4: product of pET28–CRD induced with IPTG.

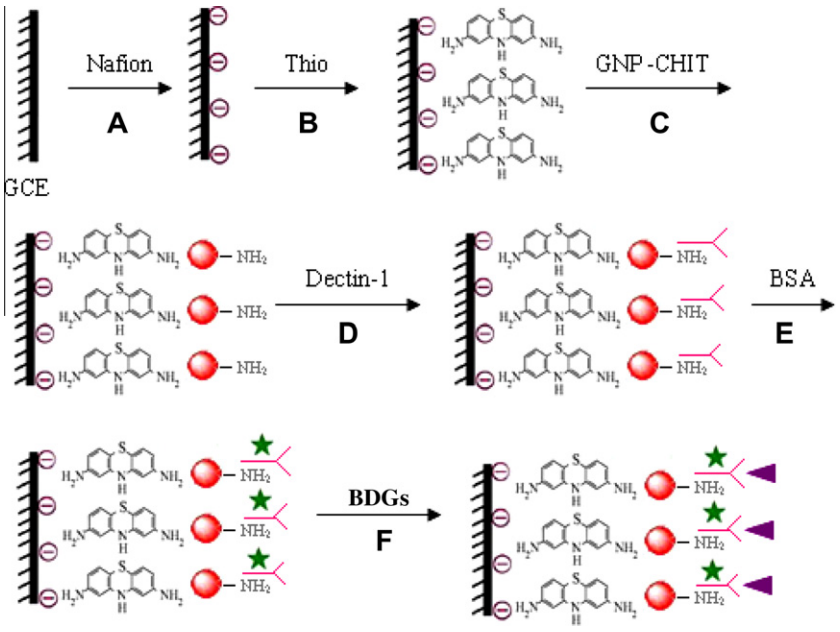

Fig. 3. Schematic illustration of the stepwise biosensor fabrication process: (A) formation of Nafion film; (B) adsorption of Thio; (C) formation of Nafion–Thio–GNP–CHIT; (D) immobilization of Dectin-1 onto the composite film; (E) blocking nonspecific active sites with BSA; (F) detection of BDGs.

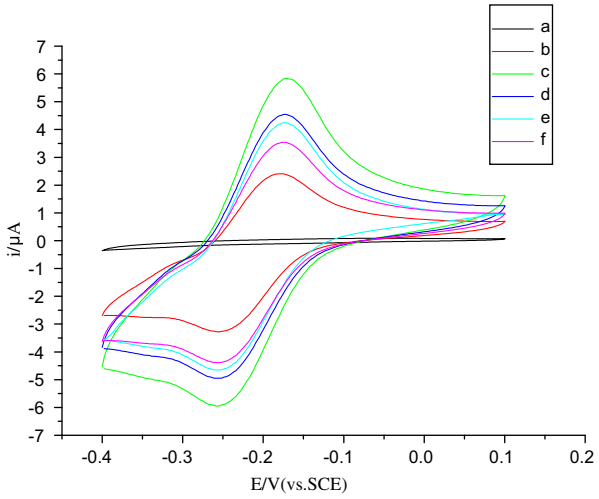

Fig. 4. Cyclic voltammograms of the different modified GCEs in the different conditions at 50 mV/s: (a) Nafion modified GCE; (b) Nafion–Thio modified GCE; (c) Nafion–Thio–GNP–CHIT modified GCE; (d) Nafion–Thio–GNP–CHIT–Dectin-1 modified GCE; (e and f) Nafion–Thio–GNP–CHIT–Dectin-1–BSA modified GCE as the biosensor in 0.1 M PBS (pH 6.5) (e) and in 0.1 M PBS (pH 6.5) after the biosensor was incubated with 50 ng/ml BDGs for 60 min (f). All potentials are given versus SCE. (For interpretation of the references to color in the key within this figure, the reader is referred to the Web version of this article.)

cal anodic and cathodic peak was observed, showing that a quasi-reversible redox reaction occurred in the electrode. The explanation was that Thio, which is a kind of phenoxazine dye and has good conductivity to transfer electron and high electrochemical signal, can be absorbed by Nafion. The peak's currents increased dramatically after GNPs were introduced into Nafion–Thio film (voltammogram c). This may be attributed to GNPs, which can act as an electron conducting tunnel or conducting wire to facilitate electron transfer [25,26]. However, the peak currents decreased after Dectin-1 was immobilized onto the Nafion–Thio–GNP–CHIT modified electrode (voltammogram d). The explanation was that the amine groups of CHIT have the capability for immobilization of the protein and the protein immobilized in the composite films can partly hinder the electron transfer. Subsequently, to avoid the nonspecific adsorption, the modified biosensor was incubated with BSA, resulting in the further decrease of peak current (voltammogram e). The explanation was that BSA can block possible remaining active sites and further hinder the electron transfer. After the biosensor was incubated with 50.0 ng/ml BDG solution, the anodic and cathodic peak current decreased dramatically (voltammogram f). The explanation was that during the incubation process, Dectin-1 immobilized onto the electrode could specifically recognize BDGs in solution to form the ligand–receptor complexes that further impede the transmission of electrons. These results strongly suggest that the BDGs can interact with immobilized CRD of Dectin-1, and this interaction can be measured by the reduction of peak current in the biosensor.

Optimization of biosensor components

Because it was demonstrated that Thio could be absorbed by Nafion through ion exchange, the content of Nafion and Thio could influence the performance of the electrode. During the ion exchange between Nafion and Thio, the quantity of Thio can be confirmed by controlling the scan number of cyclic voltammetry. With the scan proceeding, the anodic peak current and cathodic peak current rose until they reached the steady state after the cyclic scan of 20 times, showing that the immobilization of Thio onto Nafion reached the saturation state. Based on the result, the quantity of Thio can be confirmed.

To study the effect of the content of Nafion on the performance of the electrode, we investigated the content of Nafion varying from 1 to 7 μl. The response current increased with Nafion increasing from 1 to 5 μl, reaching a maximum current at 5 μl of Nafion. However, when the content of Nafion was more than 5 μl, the current intensity was found to decrease. This is likely due to the fact that an increase of Nafion could lead to an increase of the active sites in which Nafion can react with Thio, but the excessive Nafion would cause the formation of the thick film, which can hinder the electron transfer. Accordingly, 5 μl was chosen as the optimal content of Nafion.

We also attempted to optimize the GNP–CHIT content for a better signal-to-noise ratio. The response current increased with increasing proportions of GNPs to CHIT, likely due to the increased electrocatalytic sites within the composite film. However, when the proportion of GNPs to CHIT was more than 1:1 (v/v), a decrease of the response current occurred and may be attributed to the leakage of GNPs from the composite films. Based on these results, GNP–CHIT (1:1, v/v) was used in the following experiments.

Optimization of BDG assay conditions

The effect of pH on the biosensor response was investigated in the pH range of 6.0 to 8.0 in PBS with constant BDG concentrations. As shown in Fig. 5A, the current intensity increased with the solution pH increasing from 6.0 to 6.5, reaching a maximum current at pH 6.5. However, when the pH was more than 7.0, the response current decreased. It is possible that the stability of the ligand–receptor complex in weak acidic medium is higher than that in neutral medium or weak alkaline medium. Accordingly, pH 6.5 was chosen as the optimal value.

Because the incubation temperature was vital to activity of biomolecules, the effect of temperature on the ligand–receptor reaction was investigated. As shown in Fig. 5B, with increasing incubation temperatures from 20 to 50 °C, the biosensor after incubation for 60 min showed a maximum response current at 35 °C. However, when temperatures were higher than 35 °C, the response current decreased. This was most likely because high temperatures would bring about the partial denaturation of the protein. Accordingly, 35 °C was chosen as the optimal incubation temperature.

The incubation time also influences the biosensor response for BDGs. The biosensor was incubated with 50.0 ng/ml BDGs for the different times at 35 °C. As shown in Fig. 5C, the response current increased with incubation time and reached a plateau at 60 min. Longer incubation times did not cause further increases of the response current. Therefore, 60 min was chosen as the optimal incubation time for subsequent experiments.

Analytical curve for BDGs

With the optimized experimental conditions, the developed biosensor was used to detect the BDGs of the different concentrations. The response current was proportional to the BDG concentration in the linear range at concentrations from 0.5 to 100 ng/ml, with a correlation coefficient of 0.9921 (Fig. 6). The detection limit of the biosensor was calculated as 0.04 ng/ml at a signal-to-noise ratio of 3. Because a serum BDG level of 60 pg/ml was chosen as the cutoff [27], higher serum BDG levels could be detected.

Regeneration and stability

Regeneration of the biosensor was a key factor in its application and development. The prepared biosensor could be regenerated by simply immersing it in a 5-mol/L urea solution for approximately 10 min and removing it to wash with water after each detection for 60 pg/ml BDGs. A relative standard deviation of 3.7% was acquired when the electrode was repeated 10 times with consecutive measurements. After being stored at 4 °C for 10, 20, and 30 days, the response current for 60 pg/ml BDGs decreased by 1.3, 2.4, and 3.8% of the initial response, respectively. The above results

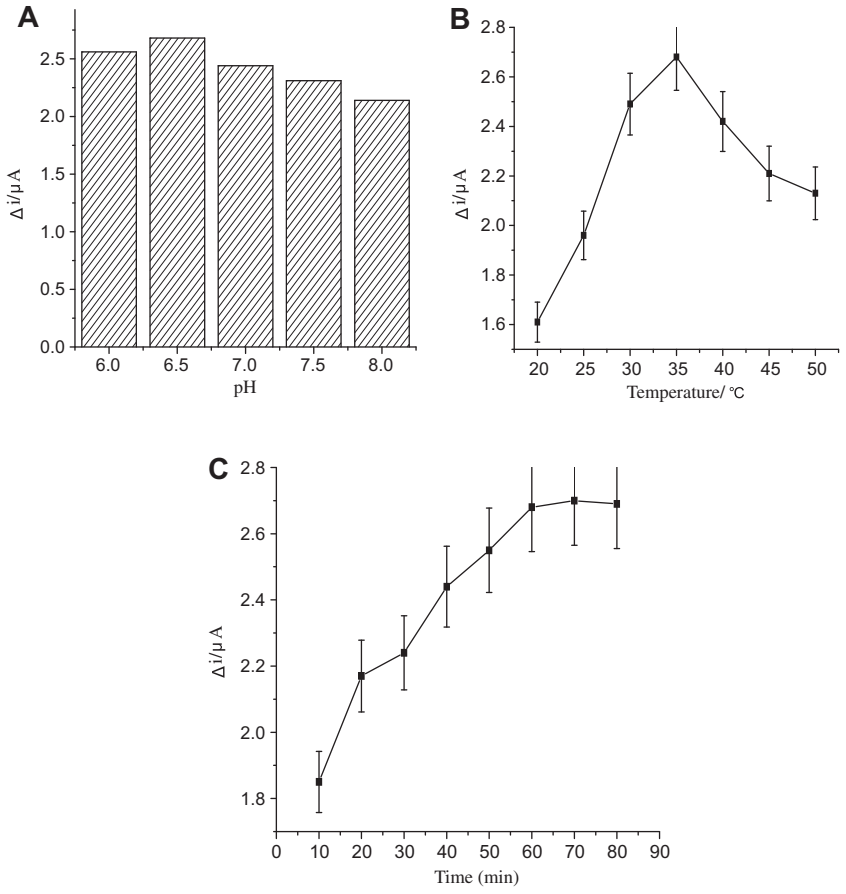

Fig. 5. (A) Effect of pH on the biosensor response. (B) Effect of incubation temperature on the biosensor response. (C) Effect of incubation time on the biosensor response.

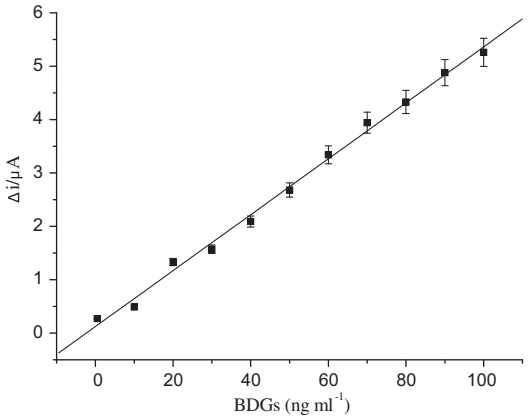

Fig. 6. Calibration curve for BDG concentrations under optimal conditions.

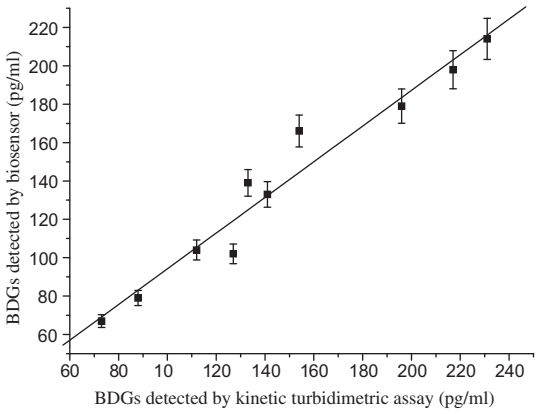

Fig. 7. Method comparison of serum BDG results obtained with turbidimetric assay and biosensor.

indicated the good accuracy, stability, and reproducibility of the biosensor.

Interference test

The polysaccharide cell wall components, which were released by the fungus into the serum during its growth in tissue, could cause interference in the accurate determination of BDGs. The influences of the interference material on the biosensor response for BDGs were investigated in 0.1 mol/L PBS (pH 6.5). As shown in Table 1, no distinguished interference current ($IC = I_2 - I_1$) is observed, where I_1 is the initial response current obtained in 0.1 mol/L PBS with 60 pg/ml BDGs and I_2 is the response current obtained in the presence of 60 pg/ml interference material in 0.1 mol/L PBS with 60 pg/ml BDGs. This may be attributed to the structural features of these polysaccharides. The results show that the biosensor demonstrated highly selective BDGs. However, hemolysis, bilirubin, and lipemia would cause the false negative results that may result from the low-level release of the BDGs of the fungi.

Recovery tests

Recovery tests were used to evaluate the accuracy of the biosensor and were performed according to National Council of Clinical Laboratory Services (NCCLS) document EP15-A [28]. Two serum samples were analyzed by kinetic turbidimetry. Then the samples were assayed with the biosensor. Table 2 shows the concentrations found and the recoveries obtained by the biosensor.

Table 1
Interference tests of biosensor response for BDGs ($n = 3$).

Interference material (60 pg/L)	Interference current (nA)
Galactan	4.1 ± 0.3
Mannan	3.4 ± 0.2
Pullulan	3.8 ± 0.3

Table 2
Determination results of BDG concentrations in serum samples ($n = 3$).

Sample	Found[a] (pg/ml)	Added (pg/ml)	Recovery (%)	Total[b] (pg/ml)
1	87	10	95 ± 3	97.8 ± 3
		50	133 ± 3	97.1 ± 3
2	121	10	136 ± 3	103.8 ± 3
		50	168 ± 4	98.2 ± 4

[a] BDG concentrations determined by turbidimetry.
[b] BDG concentrations determined by biosensor.

The results show that the biosensor demonstrates highly accurate determination of serum BDGs.

Analysis of serum samples

To illustrate feasibility of the biosensor in practical analysis, the serum BDG concentrations in 10 patients with blood cultures positive for yeast in our hospital were detected using the proposed amperometric biosensor. Moreover, the results obtained by the biosensor were compared with those obtained by kinetic turbidimetric assay (Fig. 7), which was performed according to NCCLS document EP9-A2 [29]. As shown in Fig. 7, the data were in good agreement between the two methods. The analytical curve was calibrated by the linear correlation equation $y = 1.23 + 0.93x$ ($r = 0.977$, $P < 0.0001$). Accordingly, the described method could be applied satisfactorily to the clinical determination of BDGs in human serum.

Conclusions

In this study, a recombinant Dectin-1, which could recognize and bind BDGs, was constructed by cloning and expressing the extracellular CRD of the mouse Dectin-1 gene into the a prokaryotic expression vector. A sensitive amperometric biosensor for BDGs was prepared by layer-by-layer assembly of Nafion–Thio–GNP–CHIT multilayer films on GCE surface. Detection of BDGs was accomplished by monitoring the decrease of the response current of the modified electrode. This decrease was believed to result from the formation of Dectin-1–BDG complexes that partially inhibited the transmission of electrons. The biosensor described here provides good accuracy, stability, and reproducibility as well as low cost. It could readily be used as a screening procedure for the determination of invasive fungal infections. However, one limitation of using glucan detection as an indicator for fungal infection is that the free glucan found in many medical products, such as gauze and filters, can result in the risk of false positive results. Therefore, BDG detection cannot replace other diagnostic tools, such as computer tomography imaging, in the exploration of fungal infection in high-risk patients. Perhaps a combination of a rapid biosensor-based screen and traditional microbiological culture-based detection will provide optimal specificity, accuracy, and reproducibility in clinical settings.

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