Analytica Chimica Acta 850 (2014) 85–91

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Early diagnosis of blast fungus, *Magnaporthe oryzae*, in rice plant by using an ultra-sensitive electrically magnetic-controllable electrochemical biosensor

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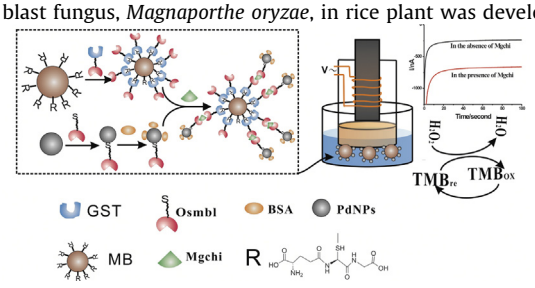
HIGHLIGHTS

- An electrochemical biosensor for the sensitive detection of *M. oryzae* in rice plant was developed.
- The *M. oryzae* in rice plant can be detected before any symptomatic lesions were observed.
- The electrochemical biosensor is simple, rapid, cost-effective and ultra-sensitive.
- The proposed biosensor can be used to help farmers timely manage the rice blast disease.

GRAPHICAL ABSTRACT

An ultra sensitive and specific magnetic-controllable electrochemical biosensor for the early diagnosis of blast fungus, *Magnaporthe oryzae*, in rice plant was developed in this study.



ARTICLE INFO

Article history:
Received 8 May 2014
Received in revised form 31 July 2014
Accepted 14 August 2014
Available online 27 August 2014

Keywords:
Magnaporthe grisea
Chitinase
Rice blast
Magnetic beads
Electrochemical biosensor

ABSTRACT

As one of the most destructive and widespread disease of rice, *Magnaporthe oryzae* (also called *Magnaporthe grisea*) has a significant negative impact on rice production. Therefore, it is still in high demand to develop extremely sensitive and accurate methods for the early diagnosis of *Magnaporthe oryzae* (*M. oryzae*). In this study, we developed a novel magnetic-controllable electrochemical biosensor for the ultra sensitive and specific detection of *M. oryzae* in rice plant by using *M. oryzae*'s chitinases (Mgchi) as biochemical marker and a rice (*Oryza sativa*) cDNA encoding mannose-binding jacalin-related lectin (Osmbl) as recognition probe. The proposed biosensor combined with the merits of chronoamperometry, electrically magnetic-controllable gold electrode and magnetic beads (MBs)-based palladium nano-particles (PdNPs) catalysis amplification, has an ultra-high sensitivity and specificity for the detection of trace *M. oryzae* in rice plant. It could be used to detect *M. oryzae* in rice plant in the initial infection stage (before any symptomatic lesions were observed) to help farmers timely manage the disease. In comparison with previous methods, the proposed method has notable advantages such as higher sensitivity, excellent specificity, short analysis time, robust resistibility to complex matrix and low cost etc. The success in this study provides a reliable approach for the early diagnosis and fast screening of *M. oryzae* in rice plant.

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http://dx.doi.org/10.1016/j.aca.2014.08.040
0003-2670/

1. Introduction

The increasing population poses enormous pressure on the current limited food-producing resources. As a main food and primary income source in many developing countries, rice production will have to increase markedly to keep pace with demand from population growth. However, the increase of rice production will have to face the decrease of cultivated land and water source [1]. It was reported by FOA (Food and Agriculture Organization of UN) that diseases, insects and weeds are responsible for about 25% failures of rice crops. As one of the most destructive and widespread diseases of rice, rice blast caused by the ascomycete fungus of *Magnaporthe oryzae* (*M. oryzae*, also called *Magnaporthe grisea*) has a significant negative impact on rice production [2,3]. It was estimated that the amount of rice destroyed by rice blast disease every year is enough to feed 60 million people [4]. In general, the blast is asymptomatic lesions in the initial infection stage (within 3 days), and it will massively propagate on the 4th day when rice was infected by *M. oryzae*. Therefore, the most effective method to save the crops from blast is timely detection of *M. oryzae* in the initial infection stage (within 3 days when rice was infected by *M. oryzae*).

Traditionally, the main technique used to identify plant pathogens is culture-based morphological observation [5,6]. However, this method usually costs a long time, about 1 week to culture and identify the pathogen, and its accuracy and reliability largely depend on the skill and taxonomical knowledge managed by analysts [7]. Therefore, it cannot be used to diagnose blast in the initial infection stage to help farmers timely control the disease. In order to overcome the limitations of morphological observation, some nucleic acid-based methods and immunoassays have been developed for the diagnoses of plant pathogens [6,8]. Especially, polymerase chain reaction (PCR) based methods are more specific, sensitive and accurate for blast diagnosis [9–12]. For example, Qi and Yang developed a method for the quantification of *M. oryzae* growth in rice plant based on DNA-based real-time PCR and RNA-based northern blot/phosphor-imaging analysis [13]. Su'udi et al. developed a sensitive method for the specific detection of *M. oryzae* by detecting as low as 1 pg *MHP1* DNA from *M. oryzae* [14], this strategy was able to analyze the amount of *M. oryzae* in the infected leaf tissues quantitatively. However, PCR-based methods have obvious disadvantages including complicated and long nucleic acid extraction procedure and relatively high assay cost etc. [15]. Especially, PCR-based methods target only a single pathogen, which make comprehensive screening of complex samples relatively unprofitable. Another sensitive and specific method used for the detection of plant pathogenic fungi is enzyme-linked immunosorbent assay (ELISA) [8,16–18]. However, the application of this method is limited by the utilization of specific antibody, since, it is very difficult to obtain species-specific antibodies of fungi.

Not long ago, stimulated by the experimental results of Dahiya et al. and Jiang et al. [19,20], we expressed and screened the *M. oryzae*'s chitinase (Mgchi) and a rice (*Oryza sativa* L., *japonica*) cDNA encoding mannose-binding jacalin-related lectin (Osmbl) with Mgchi-related gene [21]. We further demonstrated that Mgchi could be used as biochemical marker for the detection of *M. oryzae* in rice plant, and there was a specific interaction between Osmbl and Mgchi. By using the Mgchi as the biochemical marker and the Osmbl as recognition probe (receptor), we developed a novel method for the visual and specific detection of rice blast fungus, *M. oryzae*, based on the PdNPs-catalyzed TMB (3,3′,5,5′-tetramethylbenzidine sulfate)/H_2O_2 system. However, the previous method has a relatively low sensitivity, which made, it cannot be used to identify *M. oryzae* in rice plant in the initial infection stage (within 3 days when rice was infected by *M. oryzae*). Electrochemical detectors hold potential as a next-generation molecular detection strategy for the diagnostic purpose due to their high sensitivity, rapid response, low cost and excellent compatibility with miniaturization technologies [22,23]. By using the electrochemical detector, we herein developed an ultra-sensitive electrochemical biosensor for the early diagnosis of *M. oryzae* in rice plant based on an electrically magnetic-controllable electrode together with magnetic beads (MBs)-based catalysis amplification. The proposed biosensor has notable advantages including ultra-high sensitivity and specificity, simple operating process, short analysis time and low cost etc., and can be used to identify rices' *M. oryzae* in the initial infection stage to help farmers timely manage the disease.

2. Experimental

2.1. Materials and instruments

Osmbl and Mgchi were prepared with the previous method [21]. Glutathione-modified MBs (1 μm, 10 mg mL^{-1}) were purchased from Promega Company (China). The solid PdNPs were purchased from DK Nano Technology Co., Ltd. (China). TMB (3,3′,5,5′-tetramethylbenzidine sulfate)/H_2O_2 solution was purchased from Neogen Corporation (USA). The buffer solutions used in the experiment are as follows: PB buffer is the mixture of 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 solution (pH 7.4), PB-T buffer solution is the mixture of 10 mM KH_2PO_4,10 mM Na_2HPO_4 and 0.01% (w/v) Tween 20 solution (pH 7.4), the buffer solution used to extract proteins from rices' leaf is the mixture of 137 mM NaCl, 2.7 mM KCl, 10 mM KH_2PO_4, 1 mM EDTA and 0.3 mM DTT (pH 7.0). All chemicals are of analytical grade. All solutions were prepared with Milli-Q water (18.2 MΩ cm^{-1}).

M. oryzae (Wild-type strain Guy-11)-infected samples of rices' leaf (from day 1 to day 7) were obtained from Fujian Agricultural and Forestry University.

The electrically magnetic-controllable gold working electrode used in this study is the same as that previously reported [22,23]. All electrochemical measurements were performed with a CHI-660C electrochemical workstation (CH Instrument, USA). The transmission electron microscopy (TEM) image was taken with a H-9000NAR instrument (Hitachi, Japan). Zeta potential (ζ) was performed on a zeta potential analyzer (Brookhaven Instruments Corporation, USA). A gel electrophoresis (DYY-6C, Beijing LiuYi Instrument Company, China) together with a gel imaging system (Bio-Best 200E, SIM International Group Co., Ltd.) was used for the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) experiment.

2.2. Preparation of Osmbl–MBs bio-conjugates

The Osmbl–MBs bio-conjugates was prepared by immobilizing Osmbl on the surface of glutathione-modified MBs. Firstly, 5 μL of glutathione-modified MBs (10 mg mL^{-1}) was washed three times with PB buffer under the magnetic field and re-suspended in 50 μL of PB buffer. Then, about 7.5 μg (15 μL, 500 μg mL^{-1}) of Osmbl was added into MBs solution and the total was incubated for 2 h under 37 °C and gentle stirring. By this procedure, Osmbl was immobilized on the surface of glutathione-modified MBs via the strong affinity between glutathione and glutathione-*S*-transferase, since, the Osmbl was expressed and purified as a glutathione-*S*-transferase fusion protein. Subsequently, the Osmbl-modified MBs were washed for three times with PB-T buffer (pH 7.4) and re-suspended in 50 μL of PB buffer. The final solution of Osmbl-modified MBs was stored at 4 °C for next use.

2.3. Preparation of Osmbl–PdNPs bio-conjugates

PdNPs were coated with Osmbl via the interaction between cysteine or NH_3-lysine residues of Osmbl and PdNPs. Firstly, about

50 μL of 1 mg mL^{-1} PdNPs was washed three times with PB buffer by centrifuging at 10,000 rpm for 10 min, then 50 μL of 12.5 μg mL^{-1} Osmbl were added into the PdNPs solution. The total was then incubated for 2 h under 37 °C and a gentle vortex to immobilize Osmbl on the surface of PdNPs. The Osmbl-modified PdNPs was separated by centrifuging at 10,000 rpm for 20 min, and subsequently 50 μL of 1% BSA solution was added into Osmbl-modified PdNPs to block spare sites of PdNPs for 30 min at room temperature. The prepared Osmbl–PdNPs bio-conjugates was separated by centrifuging at 10,000 rpm for 20 min and washed with 50 μL of PB-T buffer (pH 7.4) for three times. Finally, the prepared Osmbl–PdNPs bio-conjugates were re-suspended in 50 μL of 1% BSA and were stored at 4 °C for next use.

2.4. Detection of Mgchi based on electrochemical measurements

About 50 μL of the prepared Osmbl–MBs bio-conjugates was firstly mixed with 200 μL of different concentration of Mgchi or sample solution, and the total was incubated for 2 h at 37 °C to capture Mgchi via the specific interaction between Osmbl and Mgchi. Then, the resulting Mgchi–Osmbl–MBs was separated and washed for three times with 200 μL of 10 mM PB-T buffer under the magnetic field. Subsequently, 50 μL Osmbl–PdNPs bio-conjugate was added into Mgchi–Osmbl–MBs and the total was incubated for 1 h at 37 °C to tag Osmbl–PdNPs to Mgchi–Osmbl–MBs conjugate via the specific interaction between Osmbl and Mgchi (see Scheme 1). The resulting PdNPs-tagged MBs was washed for five times with 200 μL of PB-T buffer, and finally the PdNPs-tagged MBs were resuspended in 25 μL PB buffer and were used for the electrochemical measurements in the TMB/H_2O_2 solution.

The electrochemical measurements were performed on a CHI-660C electrochemical workstation (Chenhua, Shanghai, China) with a three-electrode system [22,23]. An electrically magnetic-controllable gold electrode (1 mm in thickness, 3 mm in diameter) was used as a working electrode, a platinum wire was used as the auxiliary electrode and an Ag/AgCl electrode was used as a reference electrode. The working electrode was firstly polished to obtain mirror surface with 0.05 mm alumina powder, followed by sonication in water for 1 min, then it was electrochemically cleaned in 0.5 M H_2SO_4 to remove any remaining impurities and dried with nitrogen before use. Then, the PdNPs-tagged MBs obtained above was adsorbed on the surface of magnetic-controllable gold working by adding a 2 V voltage on the electrified coil and three-electrode system was immersed in 600 μL of TMB/H_2O_2 solution. The cyclic voltammetrys (CVs) scanning was performed in the potential range of 0 to +0.8 V with a scanning rate of 100 mV s^{-1}, a sample interval of 1 mV and a standing time of 2 s. The chronoamperometry was performed with incipient potential of +0.2 V, the sample interval of 0.1 s and the experimental time of 100 s.

After electrochemical measurement, by switching off the power supply of the coil, the MBs on the surface of working electrode was cleanly removed and the working electrode was used for the next detection.

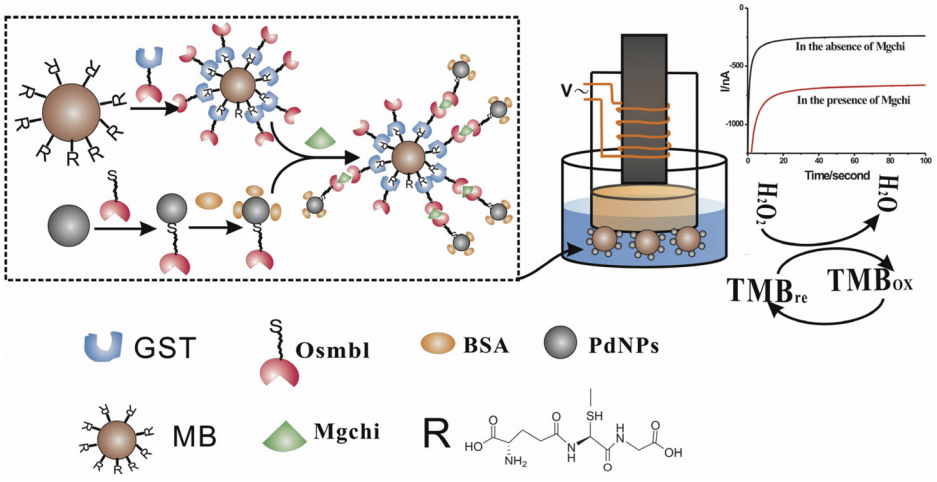
2.5. Detection of real rice plant sample

Before analysis, about 1.2 mg fresh rice leaf sample was firstly dried by liquid nitrogen freezing and crushed into fine powder with an agate mortar and pestle, then the powder was mixed with 500 μL extracting solution (the mixture of 137 mM NaCl, 2.7 mM KCl, 10 mM KH_2PO_4, 1 mM EDTA and 0.3 mM DTT, pH 7.0). The mixed solution was plenty agitated for 30 min under 4 °C to extract the total soluble protein. The mixture was then centrifuged at 12,000 rpm for 10 min to separate the deposit, and the supernatant was used for the determination of Mgchi with above method.

3. Results and discussion

3.1. The strategy of magnetic-controllable electrochemical biosensor for the detection of M. oryzae

The former research has demonstrated that Mgchi could be used as biochemical marker for the detection of *M. oryzae* in rice plant, and there was a specific interaction between Osmbl and Mgchi [21]. Therefore, the Mgchi was used as the biochemical marker of *M. oryzae* in this study, and the *M. oryzae* infection of rice was identified by detecting Mgchi in the rices' leaf. The Osmbl was used as a recognition molecule for the detection of Mgchi. The detailed principle of the work is illustrated in Scheme 1. Firstly, Osmbl, which was expressed and purified as glutathione-*S*-transferase fusion protein, was immobilized on the surface of glutathione-modified MBs to form Osmbl–MBs bio-conjugate through the strong affinity of glutathione to glutathione-*S*-transferase. At the same time, the Osmbl was immobilized on the surface of PdNPs to form Osmbl–PdNPs bio-conjugate too via the interaction between cysteine or NH_3-lysine residues and Pd. In the presence of Mgchi, Osmbl–MBs bio-conjugate can quantitatively capture Osmbl–PdNPs bio-conjugate to form sandwich-like PdNPs-tagged MBs (MBs–Osmbl–Mgchi–Osmbl–PdNPs) via the specific interaction of Osmbl and Mgchi. The resulting PdNPs-tagged MBs can be easily adsorbed on the surface of magnetic-controllable gold



Scheme 1. Schematic experimental principle of the magnetic-controllable electrochemical biosensor for detection of *M. oryzae*.

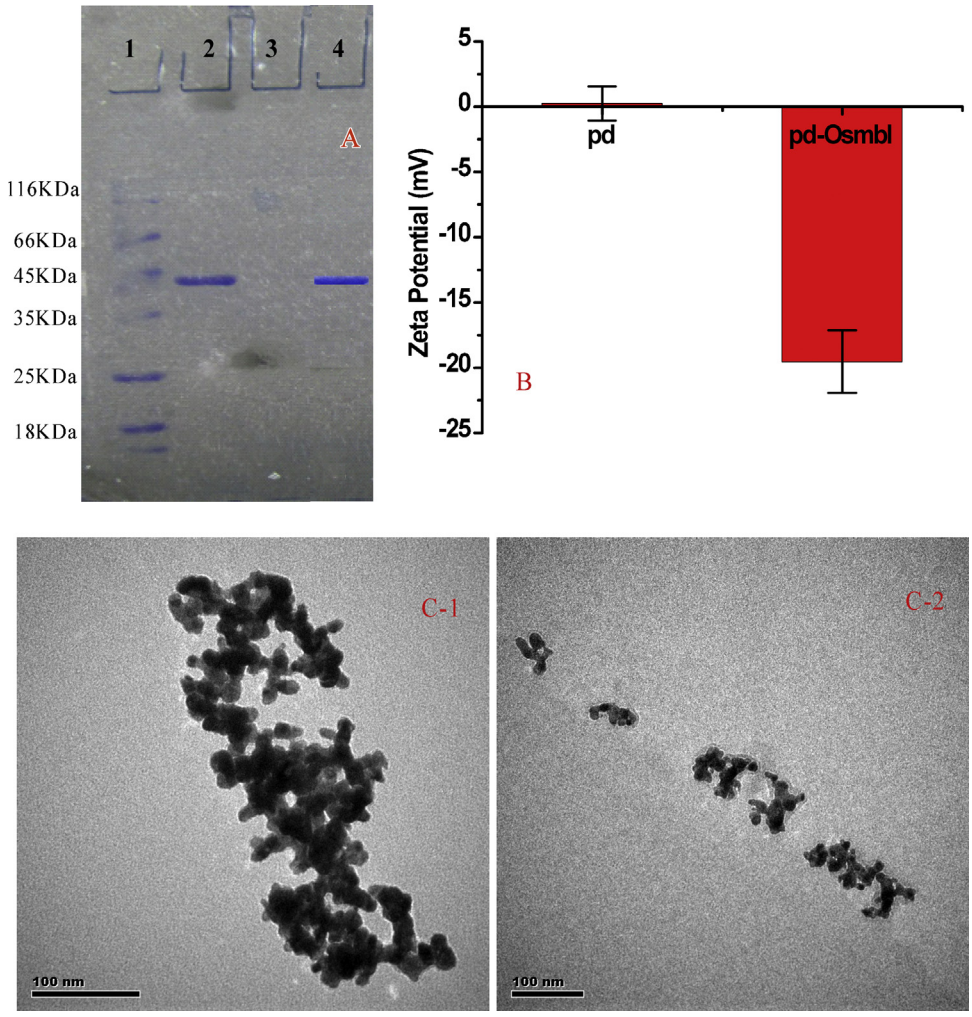


Fig. 1. Characterization of the Osmbl self-assembly on the PdNPs. (A) SDS-PAGE experimental results. Lane 1 is marker. Lane 2 is the extracting solution of Osmbl–PdNPs conjugate. The Osmbl–PdNPs conjugate was firstly dissolved in 60 μL SDS-page loading buffer (250 mM Tris–HCl – 10%, SDS – 5%, bromophenol blue – 50%, glycerol – 5%, β-mercaptoethanol, pH 6.8) for 10 min at 95 °C to extract Osmbl from Osmbl–PdNPs conjugate, and the supernatant was separated and used for the experiment. Lane 3 is the residual Osmbl solution after Osmbl was self-assembled on the PdNPs. Lane 4 is pure Osmbl solution. (B) Zeta potential of PdNPs before and after it was functionalized with Osmbl. (C) TEM image of bare PdNPs (C-1) and Osmbl–PdNPs conjugate (C-2).

working electrode and the working electrode was then used for the analysis of chronoamperometry in H_2O_2–TMB media. It is well known that PdNPs had stable peroxidase activity, it could effectively catalyze the H_2O_2-mediated oxidation of TMB [24,25]. Upon dipping the working electrode into the TMB–H_2O_2 solution, the PdNPs-tagged MBs adsorbed on the electrodes' surface catalyzed the H_2O_2-mediated oxidation of TMB, which give rise to an increasing electrochemical current signal. Therefore, the electrochemical current responding to the H_2O_2-mediated oxidation of TMB directly reflects the amount of PdNPs on the surface of working electrode, and indirectly reflects the amount of Mgchi on the surface of working electrode as well. By using this sensing platform, a simple, rapid, ultra sensitive and selective signal-on magnetic-controllable electrochemical biosensor has been developed for the early diagnosis of *M. oryzae* in rice plant.

3.2. Confirmation of the formation of Osmbl–PdNPs bio-conjugate

The formation of Osmbl–PdNPs bio-conjugate is one of the key factors of the proposed biosensor. In order to validate whether the Osmbl was successfully self-assembled on the surface of PdNPs, the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) experiment was carried out. As the SDS-PAGE image shown in Fig. 1A, the extracting solution of Osmbl–PdNPs bio-conjugate showed an obvious streak at 41 kDa (lane 2), which corresponds to pure Osmbl (lane 4). Whereas, the residue of Osmbl solution after it reacted with PdNPs showed no streak at 41 kDa (lane 3). All above SDS-PAGE experiments clearly indicated that the Osmbl has been successfully self-assembled on the surface of PdNPs. The zeta potential measurements further demonstrated that Osmbl has been successfully immobilized on the surface of PdNPs. As the results shown in Fig. 1B, the charge of bare PdNPs solution was 0.26 ± 3.33 mV, whereas the charge of Osmbl–PdNPs bio-conjugate solution is -19.52 ± 3.40 mV, which corresponds to the negatively charged Osmbl (pI 5.0) in 10 mM PB buffer (pH 7.4). The TEM images also verified the above conclusion, as shown in Fig. 1C, there was a several nanometers Osmbl and BSA self-assembly monolayer on the PdNPs surface (Fig. 1C-2).

3.3. Electrochemical characterization of the magnetic-controllable electrochemical biosensor

The cyclic voltammetrys (CVs) behavior of TMB–H_2O_2 was investigated at different stages of the biosensor preparation to validate whether the biosensor actually works as we expected. As shown in Fig. 2, two pairs of peaks ($E_{p,a1} = +0.28$ V, $E_{p,c1} = +0.34$ V,

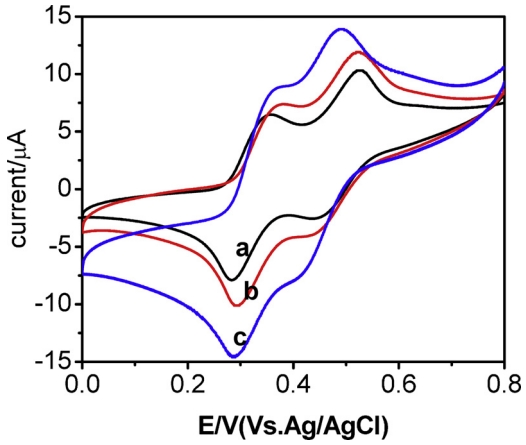


Fig. 2. Cyclic voltammetry of TMB–H_2O_2 solution at different electrode's surface. Bare electrode (black line), the magnetic-controllable gold electrode adsorbed Osmbl–MBs bio-conjugates (red line), and the magnetic-controllable gold electrode adsorbed sandwich-like PdNPs-tagged MBs (blue line). (For interpretation of the references to color in the text, the reader is referred to the web version of this article.)

$E_{p,a2}$ = +0.44 V and $E_{p,c2}$ = +0.49 V, respectively), which corresponds to two reversible one-electron couples of TMB, were observed at the bare gold electrode (Fig. 2a) [26]. In general, the oxidization of TMB with H_2O_2 is a very slow process. However, in the presence of peroxidase such as HRP, the peroxidase can effectively catalyze the H_2O_2-mediated oxidation of TMB and greatly improve the rate of the oxidation of TMB, which leads to an increasing current signal at $E_{p,a1}$ and $E_{p,c2}$ and a decreasing current signal at $E_{p,c1}$ and $E_{p,a2}$. As shown in Fig. 2b, when the Osmbl–MBs bio-conjugates are captured on the surface of the gold electrode, there is a little increase in current signal (at $E_{p,a1}$ and $E_{p,c2}$). This should be attributed to the fact that the MBs is of the intrinsic peroxidases mimetic activity and it can slightly catalyze the H_2O_2-mediated oxidation of TMB [27]. After Osmbl–MBs bio-conjugates capture Mgchi and Osmbl–PdNPs bio-conjugate to form sandwich-like PdNPs-tagged MBs, a highest current signal (at $E_{p,a1}$ and $E_{p,c2}$ respectively) was observed on the surface of the gold electrode, which adsorbed PdNPs-tagged MBs (Fig. 2c). This is because that PdNPs has peroxidase like activity and it can effectively catalyze the H_2O_2-mediated oxidation of TMB. At the same time, the solution color observed with the naked eye gradually changed from colorless to blue. The results of CVs indicated that our biosensor indeed works as we expected.

3.4. Optimization of the MBs amount, the Osmbl density on MBs' surface and the Osmbl density on PdNPs' surface

The amount of MBs absorbed on the electrode's surface and the Osmbl density immobilized on MBs' surface directly affect the analytical performance of the proposed biosensor by influencing the ability of capturing Mgchi and Osmbl–PdNPs bio-conjugate. In this study, the effect of MBs amount on the sensitivity was investigated in the range of 6.0–10 μg, and the results showed that the current signal gradually increased with the increase of the MBs amount in the range 6.0–7.5 μg, then the current signal turned to decrease with the increase of the MBs amount when the MBs amount is more than 7.5 μg (Fig. S1-A in Supporting information). This is because the ability of Osmbl–MBs bio-conjugate to capture Mgchi and Osmbl–PdNPs bio-conjugate is hindered due to the spatial restriction, when too much Osmbl–MBs bio-conjugate was absorbed on the electrode's surface. Therefore, the optimum amount of MBs absorbed on the electrode's surface is 7.5 μg.

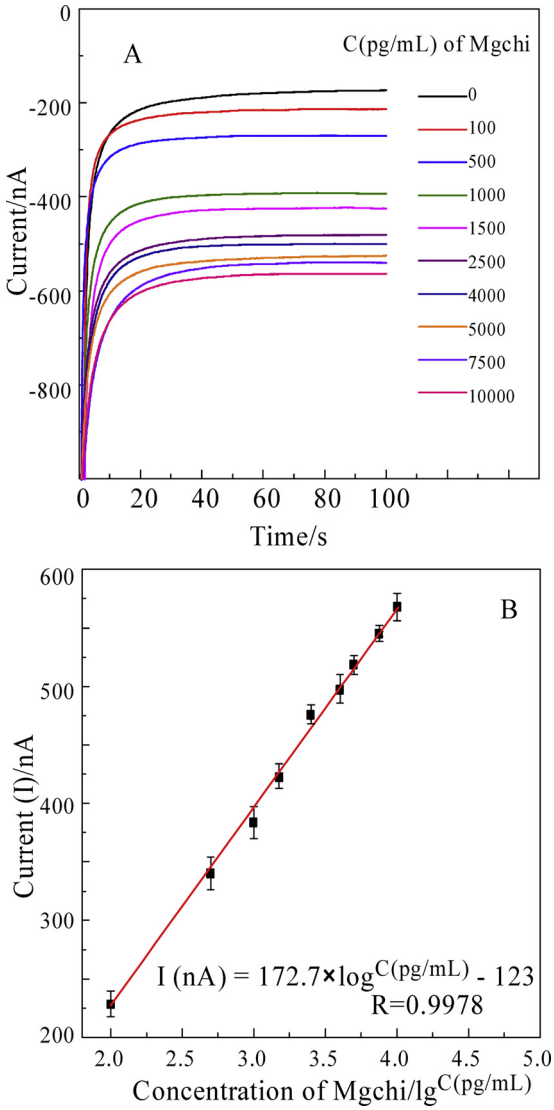


Fig. 3. A: Current–time curves of the magnetic-controllable electrochemical biosensor by detecting different concentration of Mgchi. B: The relationship between the absolute value of net current and the logarithm of Mgchi concentrations, which plotted with the data of Fig. 3A.

The Osmbl density immobilized on MBs surface was controlled by adjusting the mass ratio of MBs/Osmbl. In this study, the effect of the Osmbl density on the sensitivity was investigated by varying the mass ratio of MBs/Osmbl in the range of 1.33/1–13.3/1. As shown in Fig. S1-B (see Supporting information), the current signal became gradually larger when the mass ratio of MBs/Osmbl increased from 1.33/1 to 6.67/1, then the current signal turned to keep no change when the mass ratio of MBs/Osmbl is bigger than 6.67/1. Therefore, 6.67/1 was selected as the optimal mass ratio of MBs/Osmbl for the preparation of Osmbl–MBs bio-conjugate.

The catalytic activity of the Osmbl–PdNPs bio-conjugate would affect the sensitivity and stability of the method directly. In order to investigate the effect of the Osmbl self-assembly on the catalytic activity of PdNPs, the catalytic activity of Osmbl–PdNPs bio-conjugates was investigated in detail by incubating PdNPs in different concentration of Osmbl solution. The results shown in Fig. S1-C (see Supporting information) indicated that Osmbl–PdNPs bio-conjugates had a stable catalytic activity when PdNPs was incubated in the Osmbl solution with a mass ratio (PdNPs/Osmbl) of 80/1 for 2 h. Too long incubation time or more Osmbl would depress

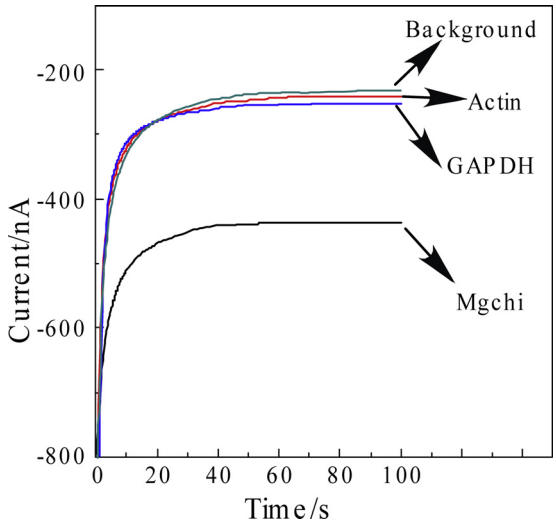


Fig. 4. Current–time curves of the magnetic-controllable electrochemical biosensor by detecting different proteins. 2600 pg mL^{-1} actin (red), 7300 pg mL^{-1} GAPDH (blue), and 2600 pg mL^{-1} Mgchi (black). (For interpretation of the references to color in the text, the reader is referred to the web version of this article.)

the catalytic activity of Osmbl–PdNPs bio-conjugates and finally lead to a lower sensitivity.

3.5. Analytical performance of the magnetic-controllable electrochemical biosensor

Under optimal conditions, the sensitivity and dynamic range of the magnetic-controllable electrochemical biosensor was evaluated by detecting a series of Mgchi standards. The absolute value of net current signal (current after subtracting the background) of TMB/H_2O_2 measured with chronoamperometry (see Fig. 3A) showed a good linear relationship with the logarithm of Mgchi concentration in the ranges of 0.1–10 ng mL^{-1} (Fig. 3B). The correlation coefficients was 0.998, and the detection limit (3σ S^{-1}, the concentration necessary to yield a net signal equal to three times the standard deviation of the background) was calculated to be 17 pg mL^{-1} (4.2×10^{-13} M). The relative standard deviations (RSD) of the biosensor for the detection of 250 pg mL^{-1} (6.1×10^{-12} M) of Mgchi is 4% ($n = 5$).

To verify the specificity of the developed method for Mgchi detection, we challenged the system with other two control proteins, *beta actin* and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which widely and abundantly existed in almost all tissues [28]. As shown in Fig. 4, in comparison with Mgchi, almost

Table 1
The analytical results of Mgchi in healthy rices' leaf and *M. oryzae*-infected rices' leaf.

Sample	Infection time (days)	Symptom	[a]Mgchi concentration (μg g^{-1})	
			Our method	[b]ELISA
Healthy rice	–		–	–
M. oryzae– infected rice	1		–	–
	2		1.28 ± 0.02	–
	3		15.37 ± 0.76	–
	4		23.94 ± 0.84	25.10 ± 0.91
	5		66.78 ± 6.54	67.85 ± 2.75
	6		198.38 ± 10.49	197.16 ± 5.68
	7		193.06 ± 6.74	194.77 ± 6.66

[a] The average concentration of ten determinations.
[b] Result detected with ELISA method, which reported by Yang et al. (2013); "–"means the concentration of Mgchi is lower than detection limit.

no current signal was observed for the *actin* and GAPDH, and even their concentrations are higher than that of Mgchi. The above results clearly indicated that the proposed method has excellent specificity for the detection of Mgchi, and the components coexisting in the plant's matrix do not interfere the determination of Mgchi.

As we mentioned above, so far, various methods have been developed for the sensitive and specific detection of *M. oryzae*, such as PCR-based methods [9–14] and enzyme-linked immunosorbent assay (ELISA) [8,16–18]. PCR-based methods are more specific, sensitive and accurate for blast diagnosis, however, it has obvious disadvantages including complicated and long nucleic acid extraction procedure and relatively high assay cost etc. The application of ELISA is limited by the lower sensitivity and the utilization of specific antibody, since, it is very difficult to obtain species-specific antibodies of fungi. Not long ago, we developed a novel method for the visual and specific detection of *M. oryzae* by using the Mgchi as the biochemical marker and the Osmbl as recognition probe. However, the previous method has a relatively low sensitivity, which made, it cannot be used to identify rices' *M. oryzae* in the initial infection stage (within 3 days when rice was infected by *M. oryzae*). In comparison with the above methods, the method reported in this study has obvious analytical advantages such as, much higher or similar sensitivity, simple operating process, short analysis time and low cost etc., which makes it suitable for the early diagnosis of rices' *M. oryzae*.

3.6. Detection of real M. oryzae-infected rice samples

The reliability and application of the proposed biosensor was validated by detecting healthy rice plant and the rice plant (cultivar CO39) infected by *M. oryzae* for different days. The analytical results were shown in Table 1 together with their photographs. As the photographs shown in Table 1, no lesion was observed in rices' leaf within 3 days when rice was infected by *M. oryzae*, and only a little of small brown specks without sporulating center were observed on the 4th day. On the 5th day, lesions became more serious and necrotic sporulating spots appeared. From 6th day, broad spindle-shaped lesions with yellow margin were observed. Soluble proteins in above each rice leaf were extracted out with the process of 2.5 and detected with the proposed magnetic-controllable electrochemical biosensor. The obtained results were compared with conventional ELISA as a reference method (Table 1). As the results shown in Table 1, no Mgchi was detected in the healthy rices' leaf and rices' leaf infected by *M. oryzae* for 1 day. A concentration of 1.28 $\mu g\,g^{-1}$ Mgchi was detected in the rices' leaf infected by *M. oryzae* for 2 days, and a concentration of Mgchi bigger than 15 $\mu g\,g^{-1}$ was detected in the rices' leaf infected by *M. oryzae* for longer than 3 days. The results obtained with our method are consistent with those obtained with ELISA, indicating that the proposed method is reliable. All the above results revealed that the proposed method had excellent specificity, robust resistibility to complex matrix and a good stability, and it can be used to identify rices' *M. oryzae* in the initial infection stage (within 3 days when the rice was infected by *M. oryzae*) to help farmers timely manage the disease.

So far, various techniques have been developed for the detection of *M. oryzae* in rice plant, such as culture-based morphological observation [5,6], PCR-based methods [8–18] and so on. These methods are difficult to be used for the early detection and a fast screening of *M. oryzae* in rice plant due to their limitations such as long assay time, low sensitivity, high cost, utilization of antibody etc. The success in this study provided a promising approach for the early diagnosis and fast screening of *M. oryzae* in rice plant.

4. Conclusions

In conclusion, we herein developed a novel method for the early diagnosis and fast screening of *M. oryzae* in rice plant by using Mgchi as biochemical marker and Osmbl as recognition probe. The method combined the merits of chronoamperometry, electrically magnetic-controllable gold electrode and MBs-based PdNPs catalysis amplification, has an ultra-high sensitivity and specificity for the detection of *M. oryzae* in rice plant. It can be used to detect *M. oryzae* in rice plant in the initial infection stage (within 3 days when the rice was infected by *M. oryzae*) to help farmers timely manage the disease. These features, as well as the ease of fabrication, operational convenience, short analysis time, robust resistibility to complex matrix and low cost etc., make our method a promising candidate for the early diagnosis and fast screening of *M. oryzae* in rice plant.

Acknowledgements

The authors gratefully acknowledge NSFC (21377025, 31071654), Ministry of Education, Science and Technology Development Center (20123514110001), Fujian Provincial Department of Science and Technology (2012J01035, 2011J01073), the Program for Changjiang Scholars and Innovative Research Team in University (No. IRT1116), and National Key Technologies R & D Program of China during the 12th Five-Year Plan Period (No. 2012BAD29B06) for the financial support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aca.2014.08.040.

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