http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 8 (11): 1544-1547, 2005
ISSN 1028-8880

Thin-layer Impedimetric Biosensors for the Free-label Immunoassay

Yu-Qing Miao
College of Chemistry and Life Science,
Zhejian Normal University, Jinhua 321004, China

Abstract: In the present study thin-layer impedimetric biosensors were developed for the free-label immunoassay of Fatty Acid Binding Protein (FABP). This test based on antibody recognition was performed within a thin-layer detection cell between two pieces of conducting glass. Antibodies were immobilized by physical adsorption. The biosensor exhibited obvious change of impedimetric signals due to the adsorbed antibody and subsequently captured antigen. No label of enzyme or aurum nanoparticle is needed. The given equivalent circuit is consonant well with the impedimetric results.

Key words: Impedimetric biosensors, free-label immunoassay, conducting glasses

INTRODUCTION

The early diagnosis of Acute Myocardial Infarction (AMI) and assessment of the extent of myocardial damage is the key to the subsequent treatment that increases the patient survival rate after AMI[1]. As recently studied and reported, heart fatty acid binding protein, which appears in the blood within one to two hours after the onset of the chest pains, is the first symptom of heart infarction and is suggested as an early plasma marker of AMI. A rapid and continuous measuring method for FABP would be desirable since measurement of biochemical markers in plasma is now universally accepted as an important indicator of AMI[2-5].

Biosensors, due to their low cost, small size, possible use *in vivo* and also their rapid response time, have been extremely investigated for the development of new immunoassay methods and strategies[6-8]. In the recent years, there has been a growing interest in impedimetric biosensors. Impedimetric assay can directly monitoring the interaction between antibody and antigen. This free-label assay doesn't need the expensive biochemical reagents of enzyme or aurum nanoparticle and makes the immunoassay procedure less complex. However, only a few impedimetric biosensors have been reported compared with the amperometric biosensors[9-12], since the free-label assay cannot enlarge the reaction signal.

In order to improve the sensitivity of impedimetric immunoassay, two pieces of conducting glass were employed to develop thin-layer electrochemical cell. FABP is chosen as a model molecule. This design only needs small volume sample. Alternating current is running between the two face-to-face adjacent flat electrodes, which makes the impedimetric signal more sensitive. It also makes sure that more large area of sensing electrodes is obtained and more amount of antibody immobilized within a limited small space.

MATERIALS AND METHODS

Materials and apparatus: Human Heart Fatty Acid Binding Protein (H-FABP) and monoclonal antibody were obtained from IgCon Therapeutics (Shanghai, China). $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). PBS (phosphate buffer solution, pH 7.2) containing 10 mM phosphate buffer was used as running buffer. Unless stated, all other chemicals were prepared in PBS.

All electrochemical experiments were performed with an Autolab PGSTA30 electrochemical instrument.

Preparation of thin-layer impedimetric biosensor: Indium tin oxide (ITO)-coated conducting glasses were used to prepare thin-layer impedimetric biosensors (Fig. 1). Two-electrode system was employed here for impedimetric detection. The area of each electrode is 1×1 cm^2 and the distance between two flat electrodes is 2 mm. Sample solution was dropped into the interspace between two flat electrodes by capillary action. This design only needs small volume sample. Also the two

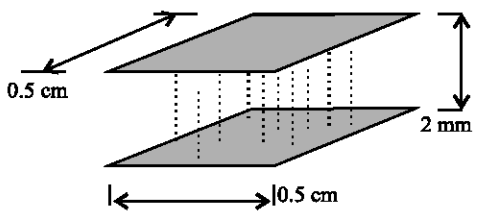

Fig. 1: Thin-layer detection cell made of two pieces of glass electrode for impedimetric detection

face-to-face electrodes are close together, which makes the impedimetric signal more sensitive.

Immobilization of antibody and detection of antigen: Two hundred microliter antibody (1 mg mL^{-1}) was dropped into the interspace between two flat electrodes and incubated at 4EC overnight. Following three rinses of electrodes in PBS to remove unbound antibody, then the electrodes were dried. Again an FABP solution, 10 μg mL^{-1} was dropped into the interspace between two electrodes and kept for 1 h. Then the electrodes were rinsed three times with PBS to remove unbound FABP.

The immobilization of antibody and capture of antigen were characterized by impedance spectroscopy over a wide range from 0.01 to 10^4 Hz, which was performed in the presence of 1 mM $K_3[Fe(CN)_6]/[K_4[Fe(CN)_6]$, 1:1 mixture, as a redox probe. A small amplitude AC (Alternate Current) potential (5 mV) was applied. Impedance spectra are plotted in the form of Nyquist plots and Bode plots. The experimental impedance spectra were simulated using electric equivalent circuits.

RESULTS AND DISCUSSION

Electrochemical Impedance Spectroscopy (EIS) is a very powerful technique for the analysis of complex electrochemical systems, which is based on the perturbation of a system at equilibrium by a small amplitude sinusoidal excitation signal (typically 5-10 mV)[13]. The impedance of the system could be measured over a wide range of AC frequencies with a single experimental procedure and so it is a sensitive indicator for a wide variety of chemical and physical properties. The components of an unknown electrochemical system could be stated by the method of equivalent circuits. Therefore it is a valuable technique for the characterization, analysis and studies of coating, batteries, fuel cells and corrosion phenomena. It has also been used extensively as a tool for investigating electrode kinetics, conducting polymers, semiconductors and sensors in animal and plant tissues and general materials etc.[14-16].

As shown in Fig. 2, at the higher frequency section, the phase degree approaches to 0 and a plateau of log (Z) can be noticed. This plateau is due to the solution resistor. With the decrease of frequency, two time variables are shown, among which one is due to the double-layer capacitance (C_{dl}) on the surface of electrode and another the Warburg diffusion. A semicircle at high frequency and a straight line at low frequency are observed in each Nyquist plot (Fig. 3). Obviously, this

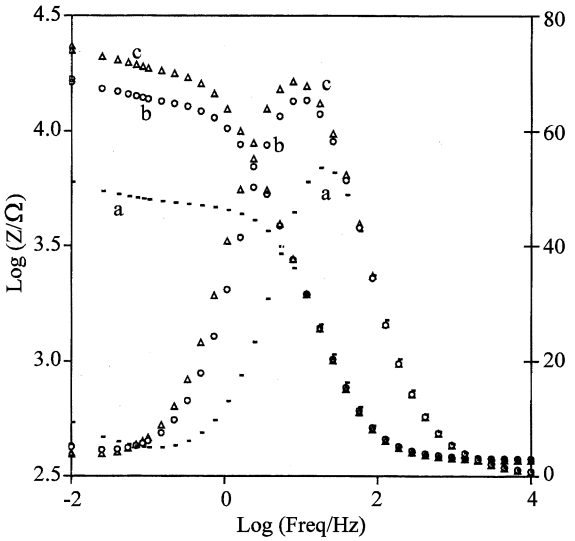

Fig. 2: Bode plots of impedance measurements in the presence of 1 mM $[Fe(CN)_6]^{3-/4-}$ at: a) the bare conducting glass electrodes, b) antibody-modified conducting glass electrodes and c) antibody-modified conducting glass electrodes with captured FABP

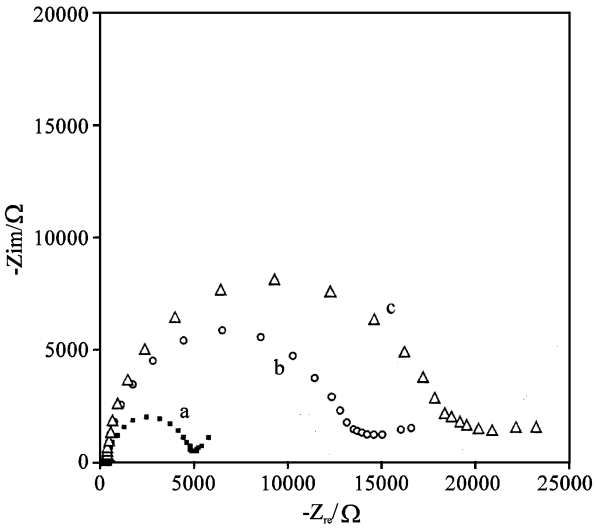

Fig. 3: Nyquist plots of impedance measurements in the presence of 1 mM $[Fe(CN)_6]^{3-/4-}$ at: a) the bare conducting glass electrodes, b) antibody-modified conducting glass electrodes and c) antibody-modified conducting glass electrodes with captured FABP

system can be modeled using the Randles circuit (Fig. 4). The semicircles at high frequency are related to the parallel combination of the double-layer capacitance and the charge transfer resistance (R_{ct}) of redox probe ($[Fe(CN)_6]^{3-/4-}$), whereas the straight lines at low frequency

Table 1: Fitted data from experimental impedance using the Rsol(Q[RctW]) equivalent circuit. The square brackets are used for elements in series and the round brackets in parallel

Fitted data		Blank electrodes	Antibody modified	FABP captured
R_{sol} (ohm)		371	379	375
Q	Y_0 (10^{-6})	3.819	4.125	4.308
	n	0.9283	0.9321	0.9371
R_{ct} (ohm)		4360	13100	18370
W		0.002697	0.001531	0.001362

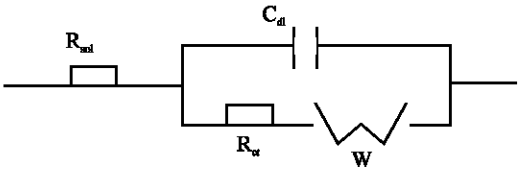

Fig. 4: Randles= circuit showing the solution resistance (R_{sol}), double layer capacitance (C_{dl}), charge transfer resistance (R_{ct}) and Warburg impedance (W)

are related to the Warburg diffusion parameter, which is used to model semi-infinite linear diffusion, that is, unrestricted diffusion to a large planar electrode. This is the simplest diffusion situation because it is only the linear distance from the electrode that matters. Figure 3 shows that the impedance is determined by slow electron transfer kinetics at high frequency, whereas the impedance is diffusion-controlled at lower frequency.

According to the results analyzed by the software of frequency response analyzer, there is a better match between the experimental impedance spectrum and the model impedance spectrum over the entire frequency range after the double-layer capacitance is replaced by a Constant Phase Element (CPE), symbolized here by Q (Table 1). The impedance spectrum is more complicated than the ideal Randles= circuit in the real electrochemical cells (Fig. 4). Here the double layer capacitance shows a phase angle of less than 90E. Such a non-ideal double layer is called CPE and its impedance is given by:

$$1/Z = Y = Q^{\circ} (j\omega)^{n}$$

When n = 1, this is the same equation as that for the impedance of ideal C_{dl}, where $Q^{\circ} = C_{dl}$.

$$1/Z = Y = j\omega Q^{\circ} = j\omega C$$

Usually n has values between 0.5 and 1. This phenomenon is supposed to be due to a number factors including surface roughness, inhomogeneous reaction rates on a surface, varying thickness or composition of a coating etc. As shown in Table 1, n is close to 1.0 and as shown in Fig. 2, the phase angle is not 90E. It means that the given CPE is approximate to an ideal double layer capacitance. According the data listed in Table 1, there is an excellent match between the experimental impedance spectrum and the model impedance spectrum over the entire frequency range

Figure 1 shows that antibody and FABP, respectively immobilized and captured onto the surface of electrodes, increase the electron transfer resistance. Here the semicircle diameter equals to the value of R_{ct}. As a result, the value of impedances at the lower frequency increase with the introduction of antibody and FABP.

The volume and concentration of antibody were optimized to make sure that all sites for antibody immobilization on the electrode surface were saturated. The introduction of BSA didn't cause non-specific response when 200 μL (mg mL^{-1}) antibody was employed. The study shows the stability of antibody immobilized by physical adsorption or hydrophobic action is excellent and satisfactory. In order to obtain a calibration curve of the biosensor, the values of log ($|Z|/|Z_0|$) were plotted versus the concentration of FABP. A frequency of 0.01 Hz was chosen since the major changes of impedance occurred at low frequency. Here $|Z_0|$ is the impedance value of antibody modified electrode before the introduction of FABP and $|Z|$ that of the subsequent electrode after FABP was captured. The thin-layer impedimetric biosensor has a linear dynamic range from 10 ng mL^{-1} to 1 μg mL^{-1}.

Conducting glasses were employed to construct the thin-layer impedimetric biosensors. Antibody can be immobilized onto the conducting glasses by spontaneous adsorption. The introduction of antibody and FABP causes the changes of impedance signal. Alternating current is running between the two face-to-face adjacent flat electrodes, which makes the impedimetric signal more sensitive. It also makes sure that more large area of sensing electrodes is obtained and more amount of antibody immobilized within a limited small space. The biosensors are possible to be used for the development of free-label immunoassay.

ACKNOWLEDGMENT

The author wishes to express his gratitude to Zhejiang Provincial Natural Science Foundation (Grant No. M203106).

REFERENCES

1. Liu, H., G.H. Dong, B. Xu, Y. Shen and H. Jing, 2005. Heart fatty acid binding protein in the rapid evaluation of myocardial damage following valve replacement surgery. Clinica Chimica Acta, 356: 147-153.

2. Pagani, F., R. Bonora, G. Bonetti and M. Panteghini, 2002. Evaluation of a sandwich enzyme-linked immunosorbent assay for the measurement of serum heart fatty acid-binding protein. Ann. Clin. Biochem., 39: 404-405.
3. Seino, Y., K. Ogata, T. Takano, J. Ishii, H. Hishida, H. Morita, H. Takeshita, Y. Takagi, H. Sugiyama, T. Tanaka and Y. Kitaura, 2003. Use of a whole blood rapid panel test for heart-type fatty acid-binding protein in patients with acute chest pain: Comparison with rapid troponin T and myoglobin tests. Am. J. Med., 115: 185-190.
4. van der Voort, D., M.M.A.L. Pelsers, J. Korf, W.T. Hermens and J.F.C. Glatz, 2003. Development of a displacement immunoassay for human heart-type fatty acid-binding protein in plasma: The basic conditions. Biosensors and Bioelectronics, 19: 465-471.
5. van der Voort, D., M.M. Pelsers, J. Korf, W.T. Hermens and J.F. Glatz, 2004. A continuous displacement immunoassay for human heart-type fatty acid-binding protein in plasma. J. Immunol. Methods., 295: 1-8.
6. Gizeli, E. and C.R. Lowe, 1996. Immunosensors. Curr. Opin. Biotechnol., 7: 66-71.
7. Ghindilis, A.L., A. Plamen, W. Michael and W. Ebtisam, 1998. Immunosensors: Electrochemical sensing and other engineering approaches. Biosensors and Bioelectronics, 13: 113-131.
8. Luppa, P.B., L.J. Sokoll and D.W. Chan, 2001. Immunosensors-principles and applications to clinical chemistry. Clinica Chimica Acta, 314: 1-26.
9. Ma, J., Y.M. Chu, J. Di, S.C. Liu, H.N. Li, J. Feng and Y.X. Ci, 1999. An electrochemical impedance immunoanalytical method for detecting immunological interaction of human mammary tumor associated glycoprotein and its monoclonal antibody. Electrochem. Comm., 1: 425-428.
10. Kharitonov, A., L. Alfonta, E. Katz and I. Willner, 2000. Probing of bioaffinity interactions at interfaces using impedance spectroscopy and chronopotentiometry. J. Electroanal. Chem., 487: 133-141.
11. Lillie, G., P. Payne and P. Vadgama, 2001. Electrochemical impedance spectroscopy as a platform for reagentless bioaffinity sensing. Sensors and Actuators B, 78: 249-256.
12. Querghi, O., A. Touhami, N. Jaffrezic-Renault, C. Martelet, H. Ben Ouada and S. Cosnier, 2002. Impedimetric immunosensors using avidin-biotin for antibody immobilization. Bioelectrochemistry, 56: 131-133.
13. Bott, A.W., 2001. Electrochemical techniques for the characterization of redox polymers. Current Separation, 19: 71-75.
14. Oliveira-Brett, A.M., L.A. Silva and C.M.A. Brett, 2002. Adsorption of guanine, guanosine and adenine at electrodes studied by differential pulse voltammetry and electrochemical impedance. Langmuir, 18: 2326-2330.
15. Omanovic, S. and S.G. Roscoe, 1999. Electrochemical studies of the adsorption behavior of bovine serum albumin on stainless steel. Langmuir, 15: 8315-8321.
16. Boubour, E. and R.B. Lennox, 2000. Insulating properties of self-assembled monolayers monitored by impedance spectroscopy. Langmuir, 16: 4222-4228.