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Layer-by-layer electrochemical biosensor with aptamer-appended active polyelectrolyte multilayer for sensitive protein determination

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ABSTRACT

Herein, we report two simple label-free electrochemical aptasensors for protein detection using layer-by-layer (LBL) self-assembled multilayers with ferrocene-appended poly(ethyleneimine) (Fc-PEI), carbon nanotubes (CNTs) and aptamer. In one sensing strategy, the Fc-PEI, CNTs and DNA aptamer are LBL assembled on the electrode surface via electrostatic interaction. In the presence of target, the aptamer on the outermost layer of the LBL self-assembled multilayer would catch the target on the electrode interface, which makes a barrier for electrons and inhibits the electro-transfer, resulting in the decreased DPV signals of Fc-PEI. Using this strategy, a wide detection range (0.3–165 ng ml^{-1}) for model target thrombin is obtained, with a low detection limit of 0.14 ng ml^{-1}. In the similar sensing strategy for detection of lysozyme, a wide detection range (0.2 ng ml^{-1} to 1.66 μg ml^{-1}) and a low detection limit (0.17 ng ml^{-1}) are obtained. These results prove that the LBL sensing strategies developed possess sensitivity, selectivity, stability and generality.

1. Introduction

Aptamers, which are novel artificially selected functional DNA or RNA oligonucleotides (Ellington and Szostak, 1990; Robertson and Joyce, 1990; Tuerk and Gold, 1990), possess high recognition ability to specific targets (Tombelli et al., 2005). Recently, numerous aptamer-based biosensors (i.e., aptasensors) for protein detection have been developed (Huang et al., 2005; Jiang et al., 2003; Liss et al., 2002; Pavlov et al., 2005; Zhang et al., 2006). Especially the electrochemical aptasensors are playing more and more important roles in this research field (Bang et al., 2005; Du et al., 2008; Katz and Willner, 2003). Most proteins possess high molecular weight and/or high charge density. When captured onto the electrode, proteins affect electron transfer on the electrode surface. This property facilitates the way to detect proteins using label-free electrochemical methods. Most of these aptasensors often utilize electrochemical impedance spectroscopy (EIS) method with a redox probe ($Fe(CN)_6^{4-/3-}$ probes) in the electrolyte (Katz and Willner, 2003; Radi et al., 2005; Xu et al., 2005). A few label-free electrochemical sensors for protein detection using voltammetry with the feasible redox probe methylene bule (MB) on the electrode surface have been reported (Bang et al., 2005; Kang et al., 2008). MB could intercalate the nucleic acid duplexes, which highly depends on the DNA conformation, the type and number of DNA bases. However, like other organic dyes, the strong nonspecific adsorption of MB sometimes could reduce the stability and repeatability of the sensor. Moreover, it can be used only in nitrogen atmosphere when the electrochemical experiment is performed. Thus, searching for effective probes attached to electrode surface for aptasensors is necessary for the label-free target detection using voltammetry measurements.

The layer-by-layer (LBL) technique (Decher, 1997) is a convenient technique for the bottom-up assembly of multilayered polymer films, because it allows the deposition of oppositely charged polyelectrolytes on solid substrates. Compared with monomolecular layers, these LBL multilayers with three-dimensional structure can bring in not only more probes to produce an amplified signal, but also more molecular recognition elements to improve the sensitivity of the detection. Besides, the stable membrane property of the multilayer make it widely used in biosensors. Recently, due to its negative charges derived from the phosphate residues in the polynucleotide chain, DNA has been gradually introduced to the LBL multilayers to provide biological activity (Sukhorukov et al., 1996). A series of electrochemical sensors have been developed using LBL techniques constructed by combining DNA with redox-active polycations (Ren et al., 2006; Rusling, 2004; Sato and Anzai, 2006). To our knowledge, only a few papers have reported the application of aptamer-based on LBL techniques

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(Sano and Shiba, 2008a, b). Recently, DeRosa and co-workers have reported when embedded in or attached to LBL polyelectrolyte matrix, aptamers could maintain their affinity and specificity for the cognate target (Sultan et al., 2009). Such an aptamer-appended polyelectrolyte multilayer could realize more applications in future aptasensor design. Thus, we construct an electrochemical aptasensor based on LBL technique for the first time.

In this work, we report two simple label-free eletectrochemical aptasensors for protein detection using LBL self-assembled multilayers on the electrode, constructed with ferrocene-appended poly(ethyleneimine) (Fc-PEI), carbon nanotubes (CNTs) and aptamer. Thrombin is a coagulation protein in the blood stream that has many effects in the coagulation cascade. Lysozyme can damage bacterial cell walls and is an important factor for protecting the tissue not to be infected with pathogens. Both of them are widely used as model targets in aptasensors for protein detection. For its property of being chemically modified easily (Beer and Cadman, 2000), Fc, the reversible redox-active probe, is assembled on the modified electrode as the signal indicator. Likewise, because carbon nanotubes (CNTs) can promote electron transfer of some electroactive substances, they have recently been widely used in the fabrication of electrochemical sensing devices and biosensors (Zheng et al., 2003). Here, we employ CNTs as an essential part of the multilayer. As shown in Fig. 1, the negatively charged indium tin oxide (ITO) electrode is modified by LBL assembly of Fc-PEI and CNTs through electrostatic interaction. Then thrombin-binding aptamer (TBA) is appended as the outermost layer to form $(\text{Fc-PEI/CNTs})_n$-(Fc-PEI/TBA) multilayer. When thrombin is present, it interacts with the TBA strand, forming thrombin-TBA complex on the electrode interface. This results in a decreased electrochemical signal of Fc, which could be used to detect the target. In a similar strategy, $(\text{Fc-PEI/CNTs/Fc-PEI/LBA})_n$ multilayer is assembled to realize the sensitive detection of lysozyme. This further proves the generality of the strategy. Such sensing strategies provide a new model for importing LBL techniques into the label-free aptamer-based sensing systems.

2. Materials and methods

2.1. Chemicals and materials

The thrombin-binding aptamer (TBA) sequence (5′ TTTTTAAAAAAAAAAAAAAGGTTGGTGTGGTTGG 3′) and the lysozyme-binding aptamer (LBA) sequence (5′ TTTTTATCTACGAATTCATCAGGGCTAAAGAGTGCAGAGTTACTTAG 3′) were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The concentrations of all the oligonucleotides were quantified using UV absorbance at 260 nm and the corresponding extinction coefficient. α-thrombin, bovine serum albumin (BSA) and lysozyme were bought from Sigma (Missouri, America). Tris(hydroxymethyl)aminomethane (Tris) was purchased from Shanghai Chemical Reagent Company (Shanghai, China). Mutil-walled carbon nanotubes (MWNTs) were bought from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China). All the DNA samples were dissolved in the Tris–HCl buffer (25 mM Tris–HCl, 300 mM NaCl, pH 8.2). Different concentrations of the proteins were prepared in the Tris–HCl buffer (T-Buffer, 20 mM Tris–HCl, 140 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, pH 7.4). DNA and protein solutions were stored at 4 °C before use. All stock and buffer solutions were prepared using Milli-Q water.

2.2. Apparatus

A normal three-electrode configuration consisting of a modified ITO working electrode (5 mm in diameter), an Ag/AgCl reference electrode, and a platinum wire auxiliary electrode was used. The cell was housed in a homemade Faraday cage to reduce stray electrical noise. Cyclic voltammetry (CV) was performed on a CHI 800 electrochemical workstation (Shanghai Chenhua Equipments, China) in the PBS solution by the potential scanning between 0 and 0.7 V. Differential pulse voltammetry (DPV) was carried out on an Autolab PGSTAT30 (the Netherlands, controlled by GPES4 and Fra software) in 100 mM PBS buffer (pH 6.5). The parameters applied were as follows: modulation time 50 ms, interval time

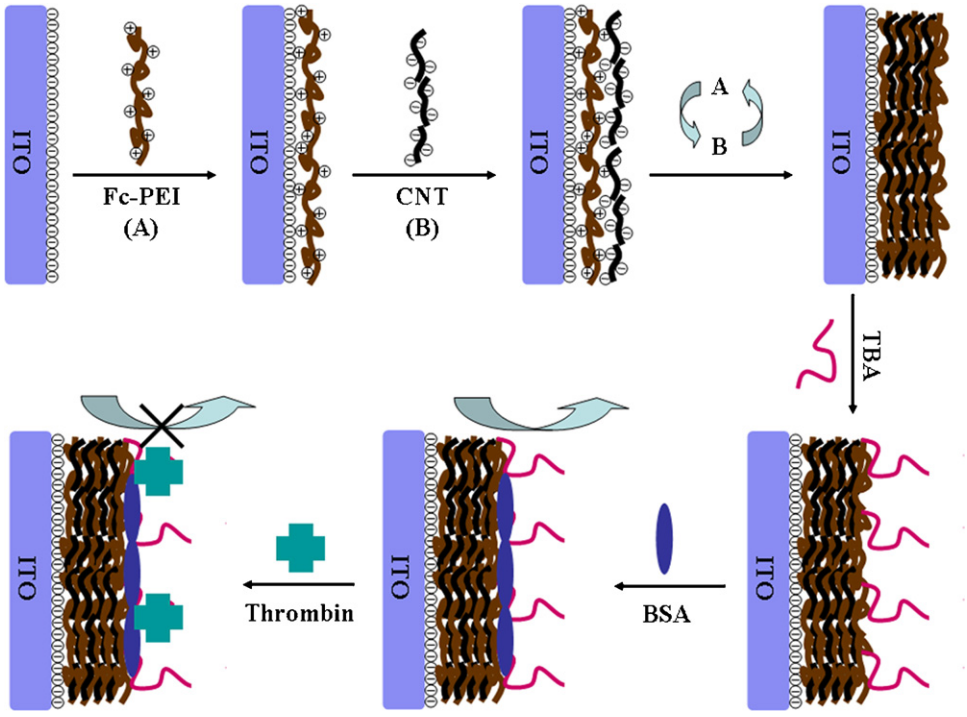

Fig. 1. Schematic diagram of eletrochemical aptasensing strategy based on LBL technique in detection of thrombin.

0.5 s, modulation amplitude 25 mV, step potential 5 mV and voltage range from 0 to 0.7 V. The raw voltammograms were treated using the Savitzky and Golay filter (level 2) included in the General Purpose Electrochemical Software (GPES) of Eco Chemie (The Netherlands) with a moving average baseline correction (Erdem et al., 2006). All measurements were carried out at room temperature (~18–22 °C). Atomic force microscopy (AFM) was performed on a SPI3800N microscope instrument (Seiko Instruments, Inc., Japan) in tapping-mode in air at ambient temperature.

2.3. Fabrication of the sensing interface

The LBL assembly process of the multilayer of (Fc-PEI/CNTs)$_5$–Fc-PEI was detailedly listed in Supporting Information. The as-prepared LBL multilayer was covered with 50 μL 1.65 μM TBA and kept over night to achieve saturation. Then the modified electrode was covered with 40 μL 1% BSA for 1 h to reduce the nonspecific adsorption. The final sensing interface was obtained after rinsing with distilled water and being dried by nitrogen.

2.4. Detection of thrombin using electrochemical measurements

The as-prepared sensing interface was measured using DPV to collect the electrochemical signal of Fc, and then immersed into a series of thrombin solutions at different concentrations (from 0.3 ng ml^{-1} to 665 ng ml^{-1}) for 1 h. After that, the decreased signal of the Fc was measured using DPV in PBS buffer. In the control experiment, the sensing interfaces were covered with T-Buffer, 50 μg ml^{-1} BSA and 50 μg ml^{-1} lysozyme solutions for 1 h, respectively. For stability detection, the LBL multilayer on the ITO electrode was dried and stored in air, and the final sensing electrode was immersed in distilled water and stored at 4 °C.

2.5. The generality of the aptamer-appended multilayers for detection of lysozyme

A similar strategy of aptamer-appended multilayer was fabricated to detect lysozyme. The LBL multilayer was composed of (Fc-PEI/CNTs/Fc-PEI/LBA)$_3$. BSA was used as a blocker to reduce the nonspecific adsorption. After the final sensing interface was obtained, it was immersed in a series of lysozyme solutions with different concentrations for 1 h, and then was measured using DPV. As a control experiment, the sensing interfaces were covered with T-Buffer, 50 μg ml^{-1} BSA and 50 μg ml^{-1} thrombin solutions for 1 h, respectively.

3. Results and discussion

3.1. Fabrication and characterization of the sensing interface

The fraction of amino groups that remained in PEI after its modification with Fc was determined using the ninhydrin test (Wei et al., 2002). The results indicated that about 28% of the PEI amino groups reacted with Fc, and the Fc-PEI still held its positive charge. In this strategy, on the negatively charged ITO electrode, the positively charged Fc-PEI was first assembled through the electrostatic interaction, followed by negatively charged CNTs assembly (Fig. 1). After recycling LBL assembly of the Fc-PEI and CNTs, more redox probes were immobilized onto the electrode interface in proportion (Fig. S1, Supporting Information). According to the standards below, we chose the bilayer numbers (n) to be 5 and with Fc-PEI as the outermost layer (n = 6) to fix the TBA via the electrostatic interaction (Fig. S1, Supporting Information). First, the electrochemical signal would provide both appropriate intensity and high sensitivity for the sensor (here, the peak current of (Fc-PEI/CNTs)$_5$-Fc-PEI

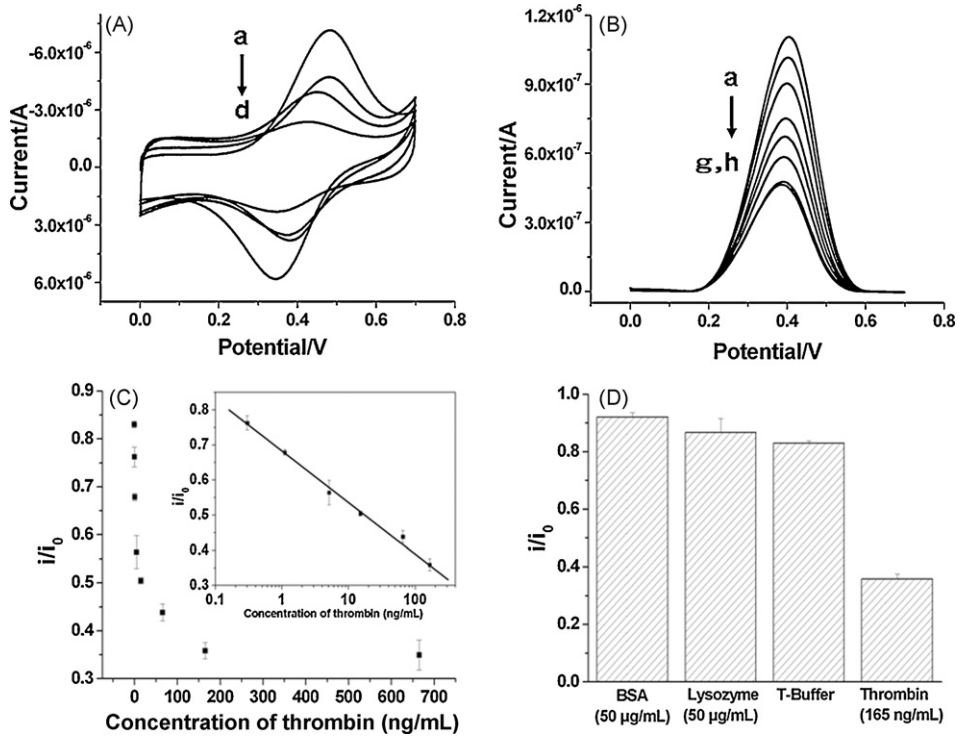

Fig. 2. (A) Cyclic voltammograms (CVs) of the self-assembled layer on the ITO electrode and combination with thrombin. The CVs from top to down: (a) the LBL multilayer, (b) 1.65 μM TBA, (c) 1% BSA and (d) 25 ng ml^{-1} thrombin. (B) The DPV responses of the sensing interface to thrombin at different concentrations. DPV response to thrombin, (a) 0 ng ml^{-1}, (b) 0.3 ng ml^{-1}, (c) 1 ng ml^{-1}, (d) 5 ng ml^{-1}, (e) 15 ng ml^{-1}, (f) 65 ng ml^{-1} (g) 165 ng ml^{-1} and (h) 665 ng ml^{-1}. (C) The relative responses of the sensor to thrombin at different concentrations (from 0.3 ng ml^{-1} to 665 ng ml^{-1}). Inset: a linear range from 0.3 ng ml^{-1} to 165 ng ml^{-1}. The error bars represent the relative standard deviation (RSD) of two measurements and the average of the RSD is 0.0178. The detections were carried out on one device and the data of each group for different concentrations of thrombin were analyzed in one day. (D) Control experiments for 50 μg ml^{-1} BSA, 50 μg ml^{-1} lysozyme, T-Buffer, and 165 ng ml^{-1} thrombin.

multilayer was about 5 μA). Second, when the thickness of the multilayer reached a certain value, it would not promote the electron transfer to the electrode surface. Besides, to make the fabrication of the sensor more simple and convenient, the whole LBL assembly process should not be too time-consuming. Otherwise, the morphology of $(Fc\text{-}PEI/CNTs)_n$ multilayer with different layer numbers were characterized using AFM (Fig. S2, Supporting Information). It was clear that the quantity of CNTs contained in multilayer was reproducible with sequential deposition. The peak current of the $(Fc\text{-}PEI/CNTs)_5$-Fc-PEI multilayer-coated electrode exhibited a linear dependence on the scan rate in the range of 10–100 mV s^{-1}, which confirmed the surface-confined species as the origin of the redox reaction (Fig. S3, Supporting Information).

The assembly steps of the sensing interface were first investigated by CV measurements. In Fig. 2A, the multilayer of $(Fc\text{-}PEI/CNTs)_5$-Fc-PEI showed a large peak current of Fc. After assembling TBA, the response was reduced obviously due to the decreased electron transfer capability of the modified electrode. At pH 7.4, the BSA (isoelectric point = 4.7) was slightly negatively charged (Chun and Stroeve, 2002). Thus, it could take up the positively charged site of Fc-PEI to reduce the nonspecific adsorption of the negatively charged thrombin. This also led to further decrease of CV signal. By immersing the prepared electrode into thrombin solution, the CV response continued to decrease, which indicated that the thrombin-TBA complex formed still remained on the electrode, inducing the blocking of the electrode surface by bulky protein and insulated the conductive support and hindered the transport of electrons toward the electrode surface.

AFM was performed to further characterize the morphology of the $(Fc\text{-}PEI/CNTs)_5$-Fc-PEI multilayer and the combination of thrombin and TBA on the multilayer. Fig. 3A exhibited AFM image of the bare ITO electrode surface. By LBL assembling, the multilayer showed a significant change of the surface morphology. The assembled CNTs on the electrode surface were uniformly distributed (Fig. 3B). After the functional sensing interface was immersed in thrombin, the surface morphology changed further (Fig. 3C). This indicated that the thrombin was bound with TBA, and the detection of thrombin could be successfully performed.

3.2. Sensitivity of thrombin detection

For thrombin detection, 1 h was used in the present experiment to make complete interaction between thrombin and TBA (Li et al., 2008). The binding of thrombin and TBA resulted in suppressing electrochemical reaction of the Fc attached to the electrode surface. A significantly decreased peak current for Fc oxidation was observed by DPV measurements. It showed that the TBA strands attached on the multilayer would catch thrombin, which made a barrier for electrons. In fact, with the concentration of thrombin increasing, the signals of Fc decreased significantly (Fig. 2B). When the concentration of thrombin increased to 665 ng ml^{-1}, the DPV signal no longer decreased, indicating the coverage of thrombin on the TBA modified electrode had reached a maximal limit.

In Fig. 2C, i/i_0 was used to evaluate the DPV anodic peak current responding to thrombin. There was a linear relationship between i/i_0 and logarithm of thrombin concentration from 0.3 ng ml^{-1} to 165 ng ml^{-1} ($R^2 = 0.9980$). The detection limit was evaluated as 0.14 ng ml^{-1} (~4.7 pM) corresponding to signal/noise ratio of 3, which can be compared with the most of previous label-free aptasensors for thrombin detection (Du et al., 2008).

Human serum was used to confirm the practical applicability of this aptasensor. In 10% of human serum, different concentrations of standard solutions of thrombin were detected by DPV. i/i_0 was also used to assess the thrombin detection ability. In Fig. S4, Supporting Information, the DPV responses at sensing interface to thrombin of different concentrations in the 10% human serum exhibited the same phenomena to those in ordinary buffer but less sensitive. The lowest detectable concentration of 5 ng ml^{-1} was gotten in 10% human serum. The results definitely illuminated the potential application of this aptasensor in samples.

3.3. Selectivity, stability and reusability of the sensing interface

Two familiar proteins, BSA and lysozyme, were chosen to test the selectivity of the sensor. In Fig. 2D, there were no remarkable changes of DPV signals for detection of 50 μg ml^{-1} BSA, 50 μg ml^{-1} lysozyme, and 0 M thrombin (i.e., T-Buffer), respectively. However, the peak current was reduced significantly with 165 ng ml^{-1} thrombin. It was illuminated that other proteins could not interact with the TBA and would not interfere with the detection of thrombin. It was worth noting that there was little decrease of the peak current for T-Buffer. That may be just the high ionic strength of the T-Buffer which could decrease the multilayer's stability.

In this work, the stability of the LBL multilayer was investigated. The multilayer of $(Fc\text{-}PEI/CNTs)_5$-Fc/PEI was left dried and air-exposed at room temperature over 24 days, and the peak current only decreased 7.5%, showing that the multilayer was comparatively stable. To testify the stability of the sensing interface, after it was immersed in distilled water and stored in 4 °C over 21days and then recovered to room temperature slowly, the peak current of the conserved sensing interface exhibited the same value as the sensor before cooling treatment. The peak current only increased 2.25%, which may be ascribed to the part degradation of the DNA. The sensing interface stored in this condition could still be used for detection of thrombin. Obviously, the assembled aptasensor achieved sufficient stability for detection of thrombin.

Reusability was another considerable aspect of the biosensors. The used sensing electrode was immersed in distilled water at 70 °C

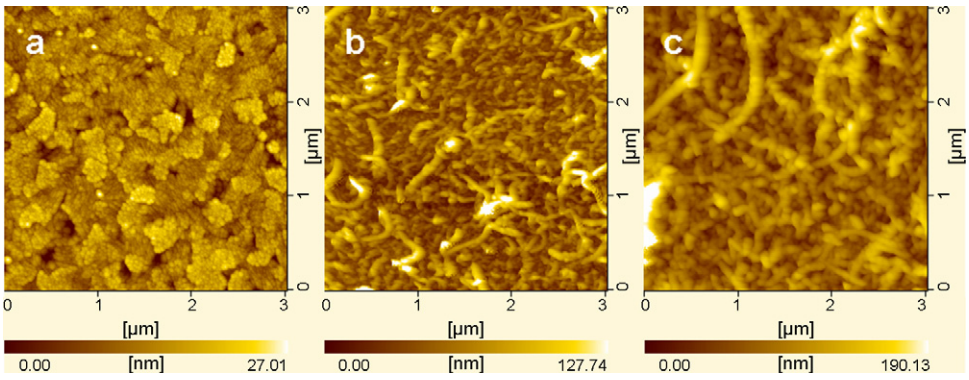

Fig. 3. Morphology of the sensing interfaces along with fabrication process characterized using AFM. The images of the bare ITO electrode (A), and the LBL multilayer on the ITO electrode (B), and the sensing interface after detected thrombin (C).

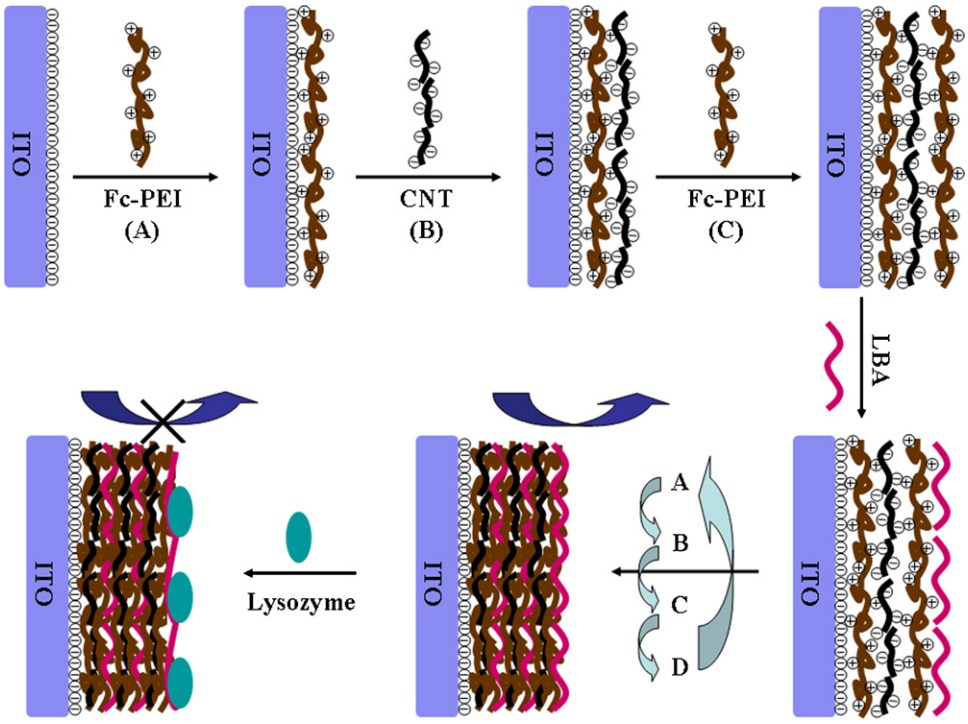

Fig. 4. Schematic diagram of eletrochemical biosensor for detection of lysozyme using aptamer-appended functional multilayers.

for 10 min to make protein degenerate. The denatured proteins could not bind to its aptamer and most of them would split from the sensing interface. Because heating may lead to decreasing the stability of this multilayer or part of the denatured proteins remain attached on the sensing interface, the DPV signal can only be regenerated up to about 73% (RSD = 0.06). And the regenerated interface could be used again to recognize thrombin.

3.4. Generality of the aptamer-appended multilayers for detection of lysozyme

As a further step, to testify the generality of the sensing strategy, we attempted to fabricate another assembly using LBA and testify the applicability of aptamer-appended multilayer. To bring more LBA on the electrode surface, a (Fc-PEI/CNTs/Fc-PEI/LBA)$_3$ multilayer was assembled instead of the multilayer of (Fc-PEI/CNTs)$_5$-(Fc-PEI/TBA) (Fig. 4). The detection principle was the same as that of aforementioned thrombin. As demonstrated in Fig. 5A, the aptamer-appended multilayer could selectively detect lysozyme and the detection limit was calculated 0.17 ng ml^{-1} based on signal/noise ratio of 3. The detection range between i/i_0 and logarithm of lysozyme concentration was from 0.2 ng ml^{-1} to 1.66 μg ml^{-1}. Using this strategy, we got a wide detection range which may be caused by more LBA in the sensing interface. It was notable that two linear relationships were obtained. In the range of lower concentration, the peak current decreased faster. Here the detection of the lysozyme also proved the controllability of the sensing surface based on LBL, which would facilitate the designing of the sensor according to researchers' different requirements.

To test the selectivity of this sensing strategy, the sensing electrodes were immersed in the 50 μg ml^{-1} BSA, 50 μg ml^{-1} thrombin, 0 μg ml^{-1} lysozyme (i.e. T-Buffer), 66.6 ng ml^{-1} lysozyme for 1 h in the same experimental conditions, respectively. Different current response signals were obtained. In Fig. 5B, the peak current of Fc oxidation was reduced significantly with only lysozyme. It was testified that these two proteins could not interact with the LBA and interfere the detection of lysozyme.

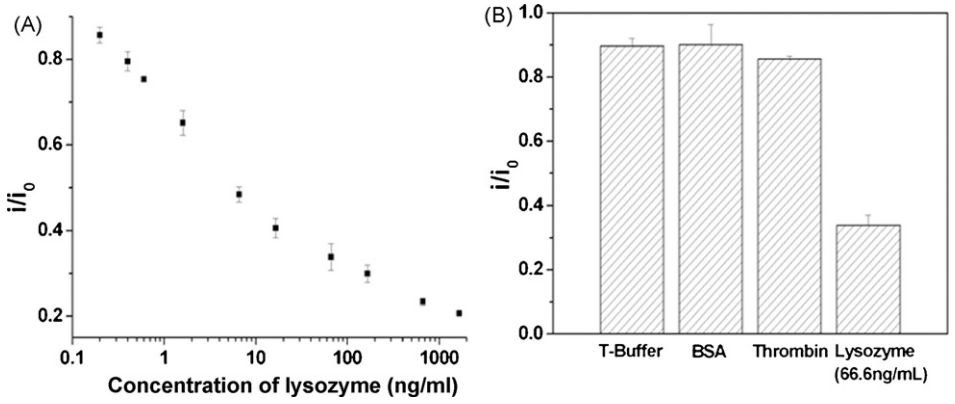

Fig. 5. (A) The relative DPV responses of the sensor to different concentrations of thrombin (from 0.2 ng ml^{-1} to 1.66 μg ml^{-1}). The error bars represent the RSD of two measurements and the average of the RSD is 0.0176. The detections were carried out on one device and the data of each group for different concentrations of thrombin were analyzed in one day. (B) Control experiment for T-Buffer, 50 μg ml^{-1} BSA, 50 μg ml^{-1} thrombin and 66.6 ng ml^{-1} lysozyme.

4. Conclusions

Two sensitive label-free electrochemical aptasensors with redox probe Fc on electrode surface for detecting proteins are developed using LBL technique, which provides a very simple and promising detection method. In one typical sensing strategy, the detection limit of thrombin is 0.14 ng ml^{-1} with the detection range from 0.3 ng ml^{-1} to 165 ng ml^{-1}. A similar strategy for the detection of lysozyme exhibits huge investigation worthiness to other assays. The as-prepared aptasensors have several advantages. (1) The Fc as a good electron transfer mediator exhibits a well defined reversible redox behavior. Compared with the DNA intercalative agent MB used in the label-free aptasensor, it is not necessary for Fc probe to be used in nitrogen atmosphere. (2) Compared with the molecular monolayer biosensors, these LBL multilayers with three-dimensional structure could bring in more redox probes and more molecular recognition elements (here, the aptamer) which could improve the sensitivity of the biosensor. Additionally, the multilayer assembly exhibits a uniform stable membrane property, which lays a good foundation as interface of label-free biosensors. (3) Such redox probe-polyelectrolyte wrapped CNTs multilayer is demonstrated as a new way to be applied in electrochemical aptasensors.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.01.003.

References

Bang, G.S., Cho, S., Kim, B.G., 2005. Biosens. Bioelectron. 21 (6), 863–870.
Beer, P.D., Cadman, J., 2000. Coord. Chem. Rev. 205, 131–155.
Chun, K.Y., Stroeve, P., 2002. Langmuir 18 (12), 4653–4658.
Decher, G., 1997. Science 277 (5330), 1232–1237.
Du, Y., Li, B.L., Wei, H., Wang, Y.L., Wang, E.K., 2008. Anal. Chem. 80 (13), 5110–5117.
Ellington, A.D., Szostak, J.W., 1990. Nature 346 (6287), 818–822.
Erdem, A., Papakonstantinou, P., Murphy, H., 2006. Anal. Chem. 78 (18), 6656–6659.
Huang, C.C., Huang, Y.F., Cao, Z.H., Tan, W.H., Chang, H.T., 2005. Anal. Chem. 77 (17), 5735–5741.
Jiang, Y.X., Zhu, C.F., Ling, L.S., Wan, L.J., Fang, X.H., Bai, C., 2003. Anal. Chem. 75 (9), 2112–2116.
Kang, Y., Feng, K.J., Chen, J.W., Jiang, J.H., Shen, G.L., Yu, R.Q., 2008. Bioelectrochemistry 73 (1), 76–81.
Katz, E., Willner, I., 2003. Electroanalysis 15 (11), 913–947.
Li, B.L., Wang, Y.L., Wei, H., Dong, S.J., 2008. Biosens. Bioelectron. 23 (7), 965–970.
Liss, M., Petersen, B., Wolf, H., Prohaska, E., 2002. Anal. Chem. 74 (17), 4488–4495.
Pavlov, V., Shlyahovsky, B., Willner, I., 2005. J. Am. Chem. Soc. 127 (18), 6522–6523.
Radi, A.E., Sanchez, J.L.A., Baldrich, E., O'Sullivan, C.K., 2005. Anal. Chem. 77 (19), 6320–6323.
Ren, K.F., Ji, J., Shen, J.C., 2006. Biomaterials 27 (7), 1152–1159.
Robertson, D.L., Joyce, G.F., 1990. Nature 344 (6265), 467–468.
Rusling, J.F., 2004. Biosens. Bioelectron. 20 (5), 1022–1028.
Sano, K.I., Shiba, K., 2008a. MRS Bull. 33 (5), 524–529.
Sano, K.I., Shiba, K., 2008b. J. Mater. Res. 23 (12), 3236–3240.
Sato, H., Anzai, J., 2006. Biomacromolecules 7 (6), 2072–2076.
Sukhorukov, G.B., Montrel, M.M., Petrov, A.I., Shabarchina, L.I., Sukhorukov, B.I., 1996. Biosens. Bioelectron. 11 (9), 913–922.
Sultan, Y., Walsh, R., Monreal, C., DeRosa, M.C., 2009. Biomacromolecules 10 (5), 1149–1154.
Tombelli, S., Minunni, A., Mascini, A., 2005. Biosens. Bioelectron. 20 (12), 2424–2434.
Tuerk, C., Gold, L., 1990. Science 249 (4968), 505–510.
Wei, X., Cruz, J., Gorski, W., 2002. Anal. Chem. 74 (19), 5039–5046.
Xu, D.K., Xu, D.W., Yu, X.B., Liu, Z.H., He, W., Ma, Z.Q., 2005. Anal. Chem. 77 (16), 5107–5113.
Zhang, H.Q., Wang, Z.W., Li, X.F., Le, X.C., 2006. Angew. Chem. Int. Ed. 45 (10), 1576–1580.
Zheng, M., Jagota, A., Strano, M.S., Santos, A.P., Barone, P., Chou, S.G., Diner, B.A., Dresselhaus, M.S., McLean, R.S., Onoa, G.B., Samsonidze, G.G., Semke, E.D., Usrey, M., Walls, D.J., 2003. Science 302 (5650), 1545–1548.