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SHORT COMMUNICATION

Conducting polymer-based impedimetric aptamer biosensor for in situ detection

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Abstract We have successfully developed the first prototype of an electrochemical protein biosensor using polypyrrole as the substrate and doped aptamer as the probe. The sensitivity for a specific target is 10 ng/ml. Two targets, platelet-derived growth factor and immunoglobulin E, have been tested. In both cases the sensor exhibited high specificity and the signal was found to increase as the target concentration increased.

Keywords Aptamer biosensor · Platelet-derived growth factor · Immunoglobulin E · In situ monitoring. Electrochemical impedance spectroscopy

Label-free biosensors for in situ measurement with high sensitivity and high specificity are of significant interest for the development of point-of-care medical diagnostics devices [1–3]. Electrochemical detection using electrochemical impedance spectroscopy (EIS) is advantageous because of its label-free aspect and high sensitivity. Label-free and reagentless aptamer biosensors based on EIS have been reported recently, demonstrating the proof of principle [4, 5]. Nevertheless, the detection limit (1–10 μg/ml) is still too high for many clinical applications (nanograms per milliliter) [4, 5]. Regarding impedimetric detection, sensitivity can be greatly improved by increasing the surface density of the immobilized probe and by introducing an electroactive supporting film.

In the procedures described below, electropolymerized polypyrrole was used as a supporting film, and an electrochemical mediator $[Fe(CN)_6]^{3-/4-}$ was applied during detection, both of which allow the sensor to achieve much higher sensitivity. Polypyrrole, a biocompatible and electrically conductive polymer, can be electropolymerized into a porous film with negatively charged molecules, known as dopants, immobilized along the positively charged polypyrrole backbone. In biosensing applications, polypyrrole has been used to immobilize biorecognition components as dopants in the conductive matrix for the electrochemical detection of glucose, proteins, and DNA [3, 6, 7]. Incorporating proteins such as enzymes, antigens, and antibodies within the polypyrrole film without signal amplification typically results in protein denaturation and steric hindrance for binding, resulting in low detection specificity and sensitivity [3, 7]. In contrast, doping polypyrrole with a DNA-detecting oligonucleotide resulted in improved specificity and excellent correlation between signal and concentration, because of its smaller size, higher biostability, and unique electrical properties [6, 8]. Aptamers, or molecules consisting of fragments of DNA or RNA, are selected from a random oligonucleotide library to bind specific targets such as small molecules or proteins. Thus, aptamers will also be incorporated into the polypyrrole matrix to a significant degree. Our previous experiments indicated that aptamers will maintain their binding-ready conformation with proper charge density under the appropriate electric potential [4]. With the addition of $[Fe(CN)_6]^{3-/4-}$, it is expected that the

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electron transfer resistance as well as the modulus of impedance will measurably increase upon specific protein binding, because the bound protein will significantly change the interfacial electrical properties. This detection mechanism is further illustrated in Fig. 1. The proof-of-concept experiments that we performed are described here. To the best of our knowledge, this is the first report of a conducting polymer-based aptamer biosensor for specific protein detection.

The electrodes we used in all experiments were 16-unit sensor chips purchased from Genefluidics (Monterey Park, CA, USA). For each unit, there were three electrodes, including the working electrode, the counter electrode, and the reference electrode [9]. All electrodes were gold films on a plastic chip. The reference electrode was determined to be +218 mV versus the standard calomel electrode by measuring cyclic voltammetry curves of 0.1 mM $[Fe(CN)_6]^{3-/4-}$. Chronoamperometry of +0.7 V for 240 s (Gamry, PA, USA) was applied to electropolymerize 0.1 M pyrrole in the presence of 10^{-5} M aptamer and 0.1% sodium dodecyl sulfate. The aptamer as a DNA fragment was believed to have been immobilized into the polymeric matrix during the course of the electropolymerization [8]. The surfactant sodium dodecyl sulfate has been reported to improve the repeatability and increase the porosity [10]. The immobilization of the aptamer probe was validated by applying a fluorophor-labeled complementary oligonucleotide and comparing the fluorescence intensity from microscopy (data not shown). The scanning electron microscope image (Fig. 2a) shows the porous morphology of the aptamer-doped polypyrrole. This may expose more probes for target binding in a defined geometric area than would be exposed by a smooth surface. As a simple estimation, the average diameter of the pores is around 100 nm, which is about 10 times the size of proteins. The size of the pores can be conveniently adjusted by changing the parameters of electropolymerization, such as potential, current, and duration [11].

To determine the best parameters for in situ monitoring of aptamer–protein specific binding, and in particular the necessary direct current bias potential and alternating current frequency, offline EIS characterization was carried out. We chose a platelet derived growth factor targeted aptamer sequence as our probe, which is the 35-mer oligonucleotide CAGGCTACGGCACGTAGAGCATCAC CATGATCCTG [12] synthesized by Integrated DNA Technologies (IDT; Coralville, IA, USA). PDGF is one of the important cytokines involved in neural inflammation. The early detection of these molecules can thus provide an effective method for detecting the onset of neuroinflammation caused by neural implants [4, 13]. After polymerization as described above, the working electrodes covered by the polymer were incubated with phosphate-buffered saline (PBS), 10 μg/ml bovine serum albumin (BSA), and 1 μg/ml PDGF for 2 h at room temperature, respectively, followed by a complete rinse with PBS and deionized water. Then, potentiostatic EIS was performed with electrodes soaked in PBS containing 10 mM $[Fe(CN)_6]^{3-/4-}$.

Figure 2b shows the Bode plot of three incubation conditions at 0 V versus open circuit potential. The difference in the impedance modulus of electrodes incubated with PBS and BSA is negligible over the relevant frequency range (1–100 kHz), while the electrode incubated with PDGF showed higher impedance over most frequencies, with the most prominent difference appearing at a frequency of around 14 kHz. To achieve the greatest specificity, 0 V versus open circuit potential and 14 kHz were chosen as the parameters for in situ EIS monitoring.

In situ measurements do not require time-consuming incubation and washing processes, potentially resulting in faster performance and simpler equipment. To thoroughly examine the feasibility of this detection method, two pairs of aptamer–targets were tested, including anti-PDGF aptamer with PDGF [12], and anti-immunoglobulin E aptamer with immunoglobulin E (IgE) [14]. IgE is an important molecule for the detection and diagnosis of allergies [15]. The sequence of the IgE-bound aptamer is GGGGCACGTT TATCCGTCCCTCCTAGTGGCGTGCCCC (IDT, IA, USA) [14]. For both in situ experiments, nonspecific proteins and specific targets were injected into the working buffer (PBS containing 10 mM $[Fe(CN)_6]^{3-/4-}$) sequentially. The real-time profile of the impedance modulus at 14 kHz during in situ experiments of IgE detection is shown in Fig. 3. Quantitatively, the change of the impedance modulus is defined as the difference in the value at the time point immediately before we injected the sample and that at the moment at which the signal achieved equilibrium. As can be

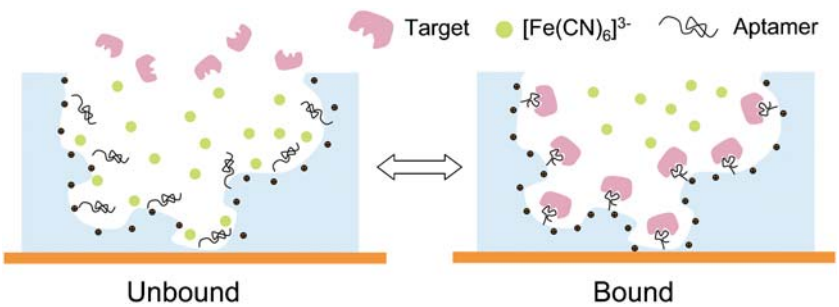

Fig. 1 Demonstration of an aptamer-coated polypyrrole (PPy) surface without and with binding of a specific protein target

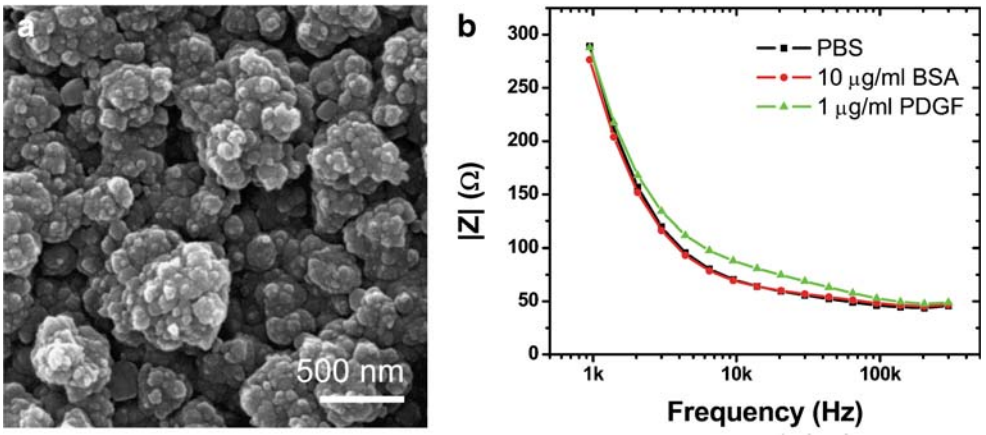

Fig. 2 **a** Scanning electron microscope image of the aptamer-doped PPy surface. **b** Bode plots of aptamer-doped PPy film incubated with phosphate-buffered saline (*PBS*), 10 μg/ml bovine serum albumin (*BSA*) and 1 μg/ml platelet-derived growth factor (*PDGF*)

seen, the injection of BSA and thrombin caused signal drops, mostly due to the change of local ion concentration. Nevertheless, the injections of the specific target IgE at different concentrations all resulted in increases of impedance compared with the negative control of BSA and thrombin. Similar results were also observed in the case of detection of PDGF (not shown). The inset in Fig. 3 shows the signal change as a function of target concentration. In both cases, impedance increases monotonously with the increase of target concentration from 10 to 10^4 ng/ml. Separate experiments were performed where target solutions were added in reverse sequence (high to low) and resulted in the same trend. The concentration profiles indicated that we can detect both IgE and PDGF in amounts as low as 10 ng/ml with sufficient specificity. These preliminary data suggest that this method is very suitable for in situ protein detection without requiring time-consuming sample preparation and washing steps.

Self-assembly monolayers (SAMs) are usually used for surface modification of gold electrodes for biosensors [16, 17]. Compared with a flat monolayer on gold, polypyrrole always has a rougher surface, and therefore more binding sites are available for probes. In addition, SAMs are usually terminated by a reactive functional group such as carboxylic acid or an amine, which requires an additional passivating step to deactivate all empty binding sites. In contrast, blocking is not necessary in polypyrrole-based biosensors, thereby significantly reducing the fabrication time. A conducting polymer-based DNA biosensor has been demonstrated successfully [8], while the protein biosensor using a direct doping strategy is still far from satisfactory in terms of specificity and sensitivity. There are two factors that may

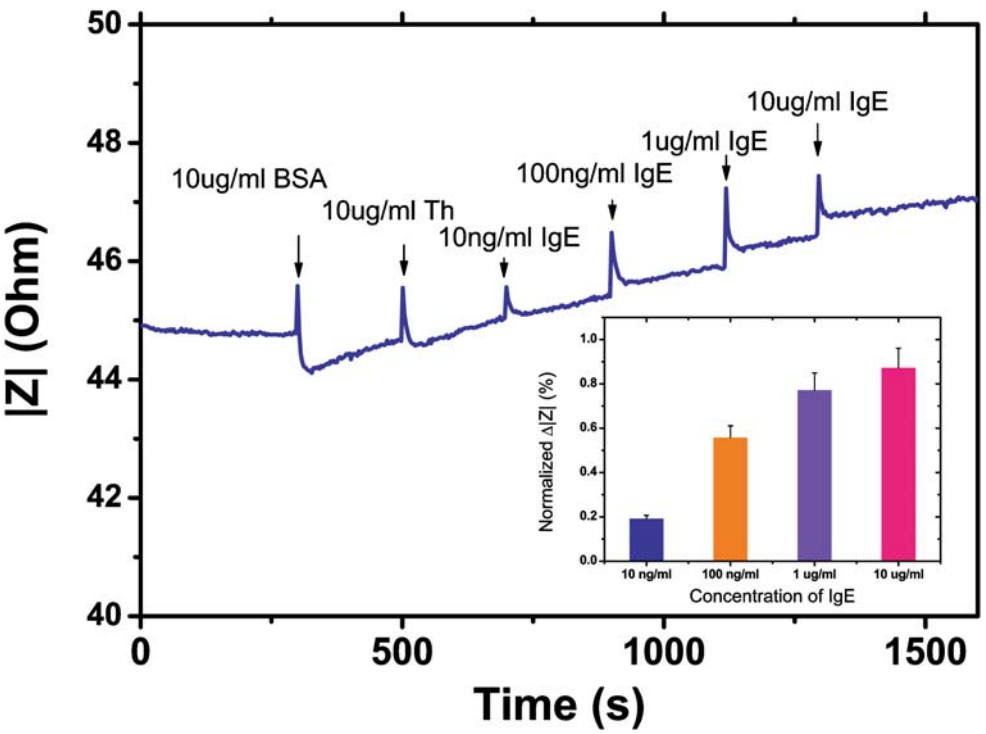

Fig. 3 Real-time monitoring of the impedance modulus. At different time points, the impact of control samples (10 μg/ml BSA and 10 μg/ml thrombin, *Th*) and specific target immunoglobulin E (*IgE*) at increasing concentrations are shown. The *inset* shows the concentration profile obtained from real-time measurements. The averaged percentage impedance modulus changes from 18 independent experiments and their standard deviations are shown

potentially lead to unsatisfactory performance. First, proteins are larger molecules with positive and negative charges distributed in different domains, resulting in low immobilization efficiency by electropolymerization. Second, proteins trapped within a polymeric matrix encounter greater steric hindrance than DNA molecules. Like DNA molecules, aptamers are small and uniformly negatively charged, and consequently serve as very suitable dopants to be incorporated into the polymer via electropolymerization. Furthermore, it is relatively easy for the aptamers to adopt the proper conformation for protein recognition.

In addition to their excellent specificity and sensitivity, biosensors incorporating electropolymerized films have extensive potential to be combined with other techniques, such as microelectromechanical systems. Gold-based electrodes can be miniaturized to the size of a few nanometers, making the production of microelectrode arrays feasible. The means to immobilize thousands of different probes onto such a microarray remains a significant challenge. Conducting polymers may provide a very convenient solution, as probes can be doped onto any microelectrode by simply applying an electrical current to the electrode in sequence. It is conceivable that this type of aptamer–polymer microarray will prominently facilitate proteomics and medical diagnostics.

Despite its great potential and future application, there are still numerous unsolved problems to be addressed. For example, the properties of a polymer film may change upon the fluctuation of the ionic concentration or the pH of the medium. Parallel detection with a control electrode is a possible solution.

In conclusion, we have successfully developed the first prototype of an electrochemical protein biosensor using polypyrrole as the substrate and doped aptamer as the probe. The sensitivity for specific targets has been improved from 1 μg/ml (without polymer) [4] to 10 ng/ml. Two targets, PDGF and IgE, have been tested. In both cases the sensor exhibited high specificity and the signal was found to increase as the target concentration increased. This novel biosensor has enormous potential for both research and clinical applications after a few improvements.

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References

1. Gerard M, Chaubey A, Malhotra BD (2002) Biosens Bioelectron 17:345
2. Liao W, Guo S, Zhao XS (2006) Front Biosci 11:186
3. Cosnier S (2003) Anal Bioanal Chem 377:507
4. Liao W, Cui XY (2007) Biosens Bioelectron 23:218
5. Rodriguez MC, Kawde AN, Wang J (2005) Chem Commun 4267
6. Wei F, Wang JH, Liao W, Zimmermann BG, Wong DT, Ho CM (2008) Nucleic Acids Res 36:e65
7. Ramanavicius A, Ramanaviciene A, Malinauskas A (2006) Electrochim Acta 51:6025
8. Wang J, Jiang M, Fortes A, Mukherjee B (1999) Anal Chim Acta 402:7
9. Gau V, Ma SC, Wang H, Tsukuda J, Kibler J, Haake DA (2005) Methods 37:73
10. Bartlett PN, Cooper JM (1993) J Electroanal Chem 362:1
11. Sadki S, Schottland P, Brodie N, Sabouraud G (2000) Chem Soc Rev 29:283
12. Yang CJ, Jockusch S, Vicens M, Turro NJ, Tan WH (2005) Proc Natl Acad Sci USA 102:17278
13. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA (2005) Ann Neurol 57:67
14. Liss M, Petersen B, Wolf H, Prohaska E (2002) Anal Chem 74:4488
15. Papamichael KI, Kreuzer MP, Guilbault GG (2007) Sens Actuators B Chem 121:178
16. Chaki NK, Vijayamohanan K (2002) Biosens Bioelectron 17:1
17. Xu DK, Xu DW, Yu XB, Liu ZH, He W, Ma ZQ (2005) Anal Chem 77:5107