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Aptamer biosensor for label-free square-wave voltammetry detection of angiogenin

Lidong Li*, Hongtao Zhao, Zhengbo Chen, Xiaojiao Mu, Lin Guo*

School of Chemistry & Environment, Beihang University, Beijing 100191, China

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ABSTRACT

Angiogenin (Ang), one of the most potent angiogenic factor, is related with the growth and metastasis of numerous tumors. This paper presents a very simple and label-free square-wave voltammetry (SWV) aptasensor to detect angiogenin, in which an anti-angiogenin-aptamer was used as a molecular recognition element, and the couple ferro/ferricyanide as a redox probe. At the bare gold electrode, the redox couple ($K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$) can be very easily accessed to the electrode surface to give a very strong SWV signal. At the anti-angiogenin/Au electrode surface, when angiogenin was added to the electrochemical cell, the binding of the analyte results in less availability for a redox reaction, which led to smaller SWV current. To quantify the amount of angiogenin, current suppressions of SWV peak were monitored using the redox couple of an $[Fe(CN)_6]^{4-/3-}$ probe. The plot of signal suppression against the logarithm of angiogenin concentration is linear with over the range from 0.01 nM to 30 nM with a detection limit of 1 pM. The aptasensor also showed very good selectivity for angiogenin without being affected by the presence of other proteins in serum. It is the first time to use a very simple method to detect the cancer marker. Such an aptasensor opens a rapid, selective and sensitive route for angiogenin detection and provides a promising strategy for other protein detections.

1. Introduction

Aptamers are nucleic acid-based molecules that can be selected to bind essentially to any molecule of choice (Ellington and Szostak, 1990; Tuerk and Gold, 1990). Compared with antibodies, aptamers possess more advantages over antibodies such as chemical synthesis, selection through the SELEX (systematic evolution of ligands by exponential enrichment) process, easy modification, high stability, target versatility, easy-to-stock, and resistant to denaturation and degradation. These properties make aptamers a promising class of agents for biomolecule detection. Aptasensors for biomolecules have been developed on the basis of different technologies, for example fluorescence (Chang et al., 2010), surface-enhanced Raman spectroscopy (SERS) (Cho et al., 2008), microgravimetric (Minunni et al., 2004), and electrochemistry (Baker et al., 2006; Zuo et al., 2007; Jin et al., 2007; Zayats et al., 2006; Willner and Zayats, 2007). Among these, electrochemical methods have attracted most attention in the development of aptasensors because of their high sensitivity, simple instrumentation, low production cost, fast response, and portability.

Angiogenin (Ang), one of the most potent angiogenic factor, a 14.4-kDa polypeptide, is a homologue of bovine pancreatic ribonuclease A (RNaseA) with a ribonucleolytic activity of four to six orders of magnitude less than that of RNaseA. Angiogenin stimulates endothelial cells to form diacyl–glycrol and to secrete prostacyclin by activating phospholipase C and phospholipase A2 (Fett et al., 1985; Bicknell and Vallee, 1988, 1989). Angiogenin also has a ribonucleolytic activity that appears to be necessary for neovascularization (Shapiro et al., 1986; St Clair et al., 1987). Tumor necrosis factor-a (TNF-(r)) induces the production and secretion of basic fibroblast growth factor in endothelial cells as well as being a chemoattractant for monocytes and a macro-phage activator (Leibovich et al., 1987; Frater-Schröder et al., 1987; Okamura et al., 1991; Beutler and Cerami, 1986). Therefore, Ang is related with the growth and metastasis of numerous tumors, it is very important to be able to assess this molecule at trace level with high sensitivity. Serum concentration of Ang is commonly detected with antibody-based enzyme-linked immunosorbant assay (ELISA) (Katona et al., 2005). In 1998, AL6, a 45 nt DNA aptamers of Ang, was generated by in vitro selection process (Nobile et al., 1998). Afterwards, Yang et al. used a single-fluorophore-modified derivative of this aptamer to detect Ang in serum based on fluorescence anisotropy (Li et al., 2007). Wang et al. developed a method by using a FRET-based aptamer probe for rapid angiogenin detection (Li et al., 2008).

* Corresponding author. Tel.: +86 010 82338162; fax: +86 010 82338162.
E-mail addresses: lilidong@buaa.edu.cn (L. Li), guolin@buaa.edu.cn (L. Guo).

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Among all the voltammetric techniques, square-wave voltammetry has many advantages, such as the excellent sensitivity and the high speed. This high speed, coupled with computer control and signal averaging, allows for experiments to be performed repetitively and increases the signal-to-noise ratio. Compared to both linear sweep and cyclic voltammetry, square-wave voltammetry has a much broader dynamic range and lower limit of detection because of its efficient discrimination of capacitance current. Applications of square-wave voltammetry include the study of electrode kinetics with regard to preceding, following, or catalytic homogeneous chemical reactions, determination of some species at trace levels (Parham and Rahbar, 2010; Nezamzadeh et al., 2007). In the square-wave voltammetry, the peak height is directly proportional to the concentration of the electroactive species and direct detection limits of redox species as low as 10 nM are possible. In addition, FIS (Faradaic Impedance Spectrometry) is also a commonly used method for probing protein binding events such as antibody–antigen interactions (Dijksma et al., 2001; Ruan et al., 2002; Bardea et al., 2000). However, FIS can be also associated with nonspecific impedance changes that could be easily mistaken for specific interactions (Bogomolova et al., 2009), that is, inability to discriminate between specific and nonspecific binding (both causing impedance increase). It has been found other factors leading to nonspecific impedance changes, such as: (i) initial electrode contamination; (ii) repetitive measurements; (iii) additional cyclic voltammetry (CV) or differential pulse voltammetry (DPV) measurements; and (iv) additional incubations in the buffer between measurements. Thus, these factors can occasionally cause very unstable signals.

Here we describe a novel label-free bioelectronic strategy for transducing aptamer–angiogenin recognition events based on recognition-induced steric-hindrance between redox probe ($Fe(CN)_6^{4-/3-}$) and the electrode surface for controlling access of redox marker ions used in SWV signal transduction. The formation of such bioaffinity complexes commonly leads to an insulating layer that retards the interfacial electron transfer kinetics between the redox probe and the electrode and increases the electron-transfer resistance. Here, we demonstrate that the aptamer–angiogenin interaction leads to an increase in the electron transfer resistance, repelling the redox marker ions to approach the electrode, leading to a substantial decrease in SWV current. The analytical technique described here only involves the utilization of a single-stranded (ss) DNA aptamer and a redox probe in solution. Thus the proposed SWV aptasensor for angiogenin is very simple, rapid, cost-effective, highly sensitive and reproducible, especially, it is lable-free, and requires no external modification on the biomolecules. Since the proposed SWV method possesses many advantages, this procedure could potentially be applied to the aptamer-based detection of other proteins and small molecules in the future. In addition, it is the first time to use a very simple method to detect the cancer marker. It provides a promising strategy for the cancer diagnosis in the future.

2. Experimental

2.1. Apparatus and reagents

A CHI 660C electrochemical workstation (Chenhua Instruments Co., Shanghai, China) was used for the electrochemical measurements. All experiments were performed using a conventional three-electrode system with a fabricated aptasensor or bare gold (diameter, 2 mm) as the working electrode, Ag/AgCl (sat. KCl) as the reference electrode, and a platinum counter electrode. All potentials were referred to the reference electrode.

The synthetic anti-angiogenin oligonucleotides, ssDNA(3′-(SH)-$(CH_2)_6$-CGG ACG AAT GCT TTG ATG TTG TGC TGG ATC CAG CGT TCA TTC TCA-5′) were obtained from TaKaRa Biotechnology (Dalian, China) Co., Ltd. Angiogenin, thrombin, lysozyme, bovine serum albumin and bovine hemoglobin form lyophilized erythrocytes were purchased from CNS Bioservices Co., Ltd. Tris–(2-carboxyethyl) phosphine hydrochloride (TCEP) was obtained from the Alfa Aesar (Tianjing) company. Tris-base was purchased from Sigma–Aldrich.

All other reagents were of analytical reagent grade. All solutions were prepared with doubly distilled water. Phosphate buffer pH 7.0 containing 1 mM $K_4Fe(CN)_6/K_3Fe(CN)_6$ was chosen as the supporting electrolyte for electrode characterization and angiogenin assays.

2.2. DNA preparation

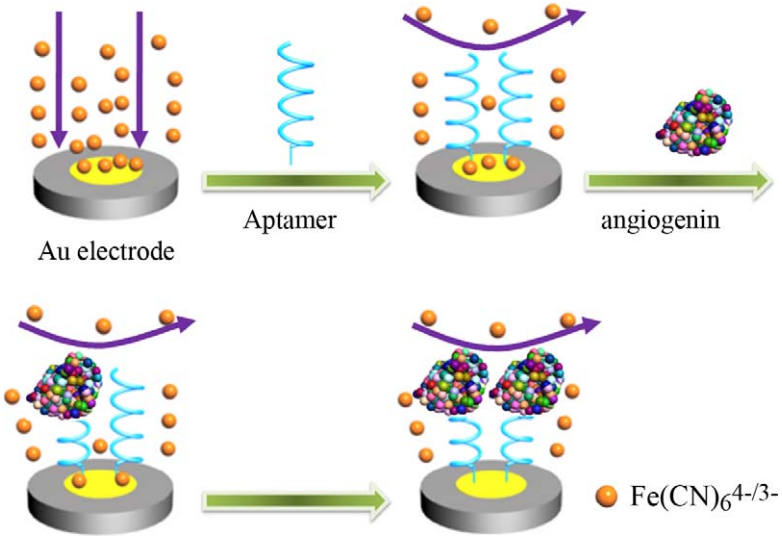
99 μL TCEP immobilization buffer (I-B: 20 mM Tris–HCl/140 mM NaCl/5 mM KCl/1 mM $MgCl_2$/1 mM $CaCl_2$ at pH 7.41) solution and 1 μL 100 μM anti-angiogenin aptamer were mixed together. Herein, TCEP was a reducer agent, which was intended to disrupt any disulphide bonds and ensure that free –SH groups are ready to react with the gold surface. Afterwards, the mixture was heated to 90 °C and allowed to gradually cool to room temperature in I-B. This heating and cooling step helps to maintain the structural flexibility of the aptamers. Afterwards, the 10 μL droplet of anti-angiogenin aptamer (1 μM in I-B) was placed on the Au electrode surface for later electrochemical analysis.

2.3. Electrode pretreatment

Prior to use, gold substrates were cleaned by immersion in a 3:1 mixture of concentrated H_2SO_4 and 30% H_2O_2 (*piranha*) for 5 min at 70 °C, followed by rinsing with copious amounts of water. *CAUTION: piranha reacts violently with organic solvents, and should be handled with extreme caution*. Afterwards, the electrode was polished with 1.0, 0.3, and 0.05 μm alumina slurry on a polishing pad consecutively. The electrode was then ultrasonically cleaned in ethanol for 1 min and in water for 2 min. The electrode was subsequently voltammetrically cycled in 0.5 M H_2SO_4 with the potential between −0.2 and +1.6 V at 0.1 V s^{-1} until a representative cyclic voltammogram of a clean gold electrode was obtained. Afterwards, the electrode was incubated in 10 μL droplet of anti-angiogenin aptamer (1 μM in I-B) for 12 h at room temperature in 100% humidity. Finally, the gold surface was rinsed again with I-B and then with deionized water, followed by drying under an N_2 stream. For detection of angiogenin, 10 μL droplets of different angiogenin concentrations in I-B were deposited on to the aptasensor and kept for 30 min at 37 °C. Non-binding angiogenin was removed by rinsing with I-B and deionized water. A schematic representation of aptasensor with fabrication steps and performance is displayed in Scheme 1.

2.4. Electrochemical measurements

All electrochemical measurements were performed in an electrochemical cell containing 10 mL of 0.2 M PBS, 1 mM $K_4[Fe(CN)_6]$–1 mM $K_3[Fe(CN)_6]$ (pH 7.0). The electrochemical square-wave voltammetry measurements were carried out under the following conditions: The voltage scanned from 0 V to 0.6 V with a potential incremental of 0.004 V, the amplitude, the frequency and the static time were kept as 0.025 V, 25 Hz, and 2 s, respectively. The Faradic impedance spectroscopy was recorded within the frequency from 100 kHz to 0.1 Hz with a sampling rate of 2 points per decade. The cyclic voltammetric experiments were



Scheme 1. Schematic diagram showing biosensor fabrication and sensing mechanism.

taken at a scan rate of 0.1 V s^{-1}. All measurements were carried out at room temperature.

3. Results and discussion

3.1. Characterization of aptasensor

In order to clarify the electrochemical properties of the resulting aptasensor, cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were used to monitor the process of fabrication of the aptasensor in each step. Fig. S1a shows characterization CVs for each fabrication step of the proposed sensor. As shown in Fig. S1a, curve 1, the $Fe(CN)_6^{4-/3-}$ redox couple showed reversible behavior at a bare gold electrode with a peak-to-peak separation ΔE_p of 67 mV. After the DNA probe was immobilized onto the surface of the gold electrode, a significant increase in the ΔE_p of the electrode was observed (Fig. S1a, curve 2), probably because of the established kinetics barrier between $[Fe(CN)_6]^{4-/3-}$ and the negatively charged phosphate backbone of the DNA. Fig. S1b shows the Nyquist plots of impedance spectra obtained at each preparation step. The bare Au electrode exhibits a very small semicircle domain, suggesting a very low electron-transfer resistance to the redox couples dissolved in the electrolyte solution. After anti-angiogenin aptamers were self-assembled on to the surface of the gold electrode, the Ret markedly increased to 2.4 kΩ (Fig. S1b, curve 2), which is again due to the created kinetics barrier between $[Fe(CN)_6]^{4-/3-}$ and the negatively charged phosphate backbones of aptamer.

3.2. Performance of the aptasensor

To quantitatively assess the detection limit and response range of the angiogenin aptasensor, Fig. 1a shows the SWV plots of aptamer/gold electrode for angiogenin with different concentrations. Fig. 1b shows the signal suppression in SWV as a function of angiogenin concentration. The signal suppression observed at a given target concentration, which is the decrease in current observed upon target binding. The signal suppression is calculated by the change in peak signal upon addition of target from the original signal observed in the absence of target. Fig. 1b displays the dependence of signal suppression on the concentration

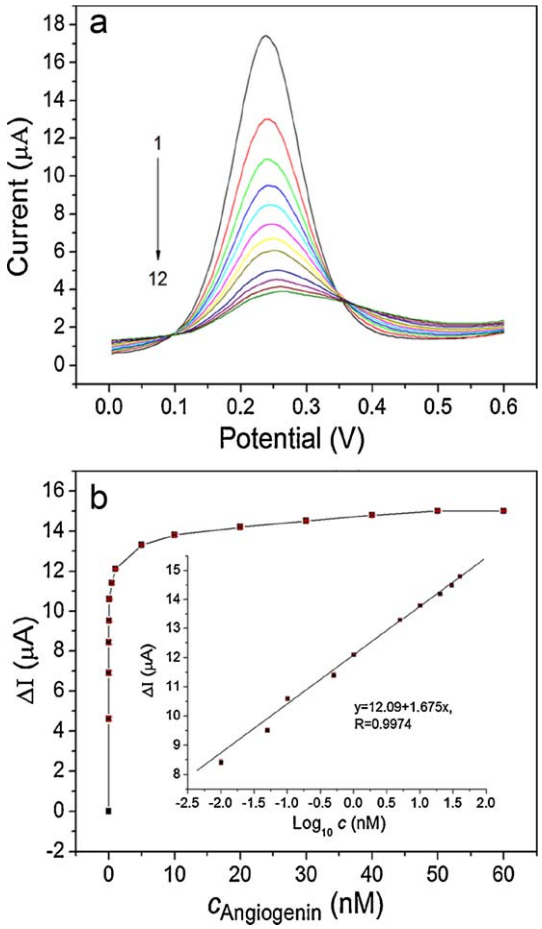


Fig. 1. (a) Square-wave voltammograms of sensing system to different concentrations of angiogenin of (1) 0 nM, (2) 0.001 nM, (3) 0.005 nM, (4) 0.01 nM, (5) 0.05 nM, (6) 0.1 nM, (7) 0.5 nM, (8) 1 nM, (9) 5 nM, (10) 10 nM, (11) 20 nM, and (12) 30 nM in I-B. (b) The signal suppression in the SWV as a function of angiogenin concentration. Inset: the SWV peak current is linear with logarithm of angiogenin concentration over the range from 0.01 to 30 nM. All electrochemical measurements were carried out in 10 mL of 0.2 M PBS, containing 1 mM $K_4[Fe(CN)_6]$–1 mM $K_3[Fe(CN)_6]$ (pH 7.0). The illustrated error bars represent the standard deviation of four measurements conducted with a single electrode at each concentration.

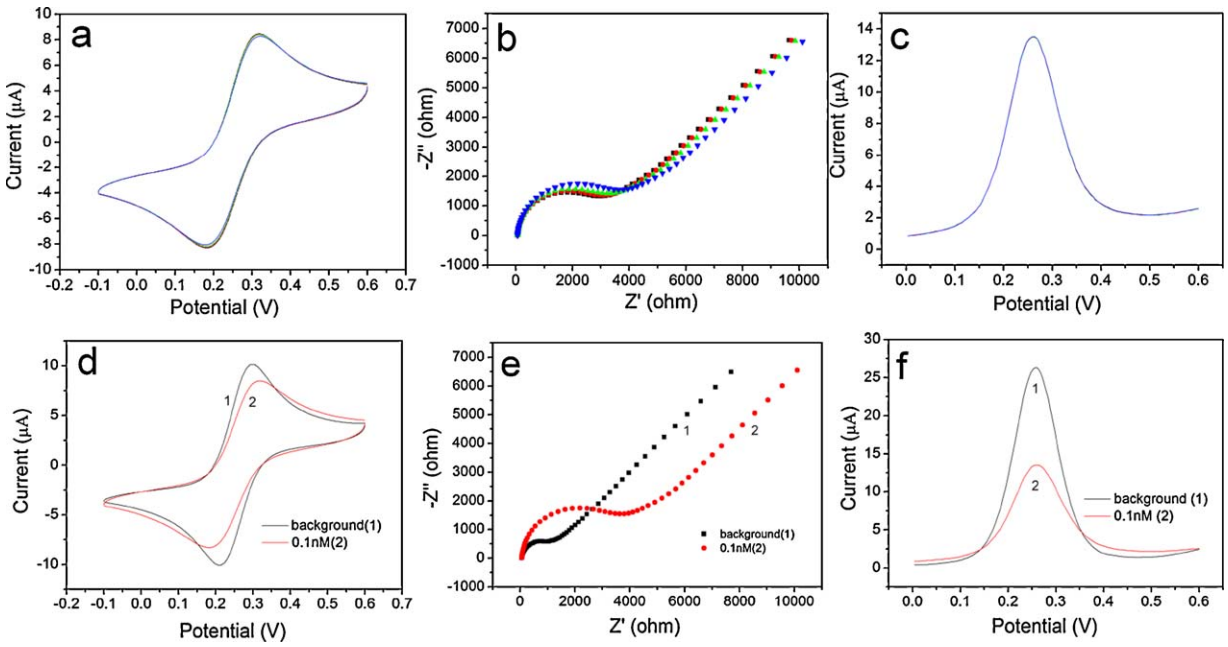


Fig. 2. Overlay of 4 consecutive repeated (a) CV measurements, (b) EIS measurements, (c) SWV measurements performed at modified electrode every 5 min in the solution with 0.1 nM addition of angiogenin. Overlay of curves of blank and the solution with 0.1 nM addition of angiogenin by (d) CV measurements, (e) EIS measurements, (f) SWV measurements. All electrochemical measurements were carried out at anti-angiogenin aptamer/gold electrode in 10 mL of 0.2 M PBS, containing 1 mM $K_4[Fe(CN)_6]$–1 mM $K_3[Fe(CN)_6]$ (pH 7.0).

of angiogenin (0.001–30 nM). The inset of Fig. 1b shows signal suppression is linear with logarithm of angiogenin concentration over a range from 0.01 nM to 30 nM. The regression equation was $\Delta I = 12.09 + 1.675 \log c$ (unit of c, nM) and the regression coefficient (R) was 0.9974. The directly measured limit of this method was 1 pM (evaluated by the actually SWV response as the lowest concentration of angiogenin that could be distinguished at a stated level of probability from the blank solutions) (Fig. 1). In order to test if the proposed aptasensor with SWV measurements is better than other electrochemical methods, a control experiment by using CV and EIS was carried out. The repeatability results with different electrochemical methods are shown in Fig. 2a–c, and the signal responses from the blank to the 0.1 nM addition of angiogenin by different methods are shown in Fig. 2d–f. From Fig. 2a, it can be seen that the CV curves of four consecutive repeated measurements with 0.1 nM angiogenin in the solution show slightly different. Obviously, in Fig. 2b, such repeated measurement-related instability of the signal is more pronounced with EIS method. One reason for measurement-related R_{et} increase is the rearrangement of the charged probe or probe-analyte complex due to the applied positive potential during AC impedance measurement. Fig. 2c shows the corresponding SWV curves of consecutive repeated measurements, four curves are perfectly matched, indicating very good repeatability of the SWV methods. The standard deviations (STD) of four successive measurements for each electrochemical method are also concluded in Table S1. As shown in Table S1, the SWV gives the smallest STD value of 0.15%, which is good enough for trace level analysis. Fig. 2d shows the CVs of both blank solution and 0.1 nM angiogenin. By addition of 0.1 nM analyte, the peak significantly shifts. Meanwhile, EIS experiments were also carried out on the same electrode (as shown in Fig. 2e). The relative signal changes are also summarized in Table S1. In addition, the required time of each measurement for different methods is also given in the table. The data indicates that SWV takes only 180 s for one measurement, which is much shorter than CV and EIS. In a word, compared with CV and EIS, the SWV method is the most repeatable, sensitive and rapid way for angiogenin analysis (Fig. 2).

3.3. Sensing mechanism of the aptasensor

The fabrication steps and the sensing mechanism to this angiogenin aptasensor are shown in Scheme 1. At the bare gold electrode, the redox couple ($K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$) can be very easily accessed to the electrode surface to give a very strong SWV signal (Fig. S2, curve 1). After the modification of anti-agniogenin aptamer, the weaker SWV peak is observed (Fig. S2, curve 2), which is attributed to the increasing kinetics barrier between $[Fe(CN)_6]^{4-/3-}$ and the negatively charged phosphate backbone of the DNA aptamer. After angiogenin was added to the electrochemical cell, the binding of the analyte resulting in less availability for a redox reaction, which led to even smaller SWV current (shown in Fig. S2, curve 3). Keep increasing the concentration of the analyte, less and less redox couples could be approached to the electrode surface, therefore, SWV current decreases as shown in Fig. 1 (curves 2–12) (Scheme 1).

3.4. The specificity of the aptasensor

The specificity of the sensor was determined by challenging it with other proteins lysozyme, thrombin, hemoglobin, at 100 nM concentrations. Importantly, the SWV signal for the other proteins was only as small as the initial background (in the presence of 0.5 nM angiogenin). However, when we added only 1 nM angiogenin, the signal decreased dramatically (as shown in Fig. 3a). In Fig. 3b, the value at the y-axis can be normalized by the current decrease for angiogenin versus the initial background, the relative signal suppression for all three other proteins is only below 2%. This excellent selectivity arises from the high specificity of the anti-angiogenin aptamer (Fig. 3).

3.5. Real sample analysis

In real sample analysis, the complexity of serum may lead to the non-specific bonding to the electrode surface and decrease the baseline signal. However, the influence of which would be the same

Table 1
Recovery rates of the proposed aptasensor.

Sample	Concentration obtained with aptasensor (nmol L^{-1})	Recovery rate (%)[a]	RSD (%)[b]
Serum + 0.01 nmol L^{-1} angiogenin	0.0921	92.1	3.52
Serum + 1.00 nmol L^{-1} angiogenin	1.16	116	4.87

[a] Recovery rate = ((spiked sample − sample)/add scalar) × 100%.
[b] RSD is relative standard deviation (n = 5).

to the blank solution and the sample with a certain concentration of angiogenin. Therefore, the signal suppression remains. Moreover, the real sample has been diluted 50 times before each trial; thus, the baseline decrease could be negligible. To investigate if the method was applicable to real samples, we tested real and spiked serum samples with different angiogenin concentrations. As shown in Table 1, the recoveries of standard addition are 92.1% and 116% for angiogenin concentrations of 0.01 nM and 1 nM, respectively, when using the proposed electrochemical method. The RSD values of five successive measurements are also shown in Table 1. In order to provide comparative data with a commercially available method, we also analyzed four serum samples of lung cancer patients using the Human Ang Immunoassay (Quantikine, Catalog Number DAN00). The experimental process was operated according to operating protocol (http://www.rndsystems.com/pdf/DAN00.pdf). The results were presented in Table 2. As shown in Table 2, the percent

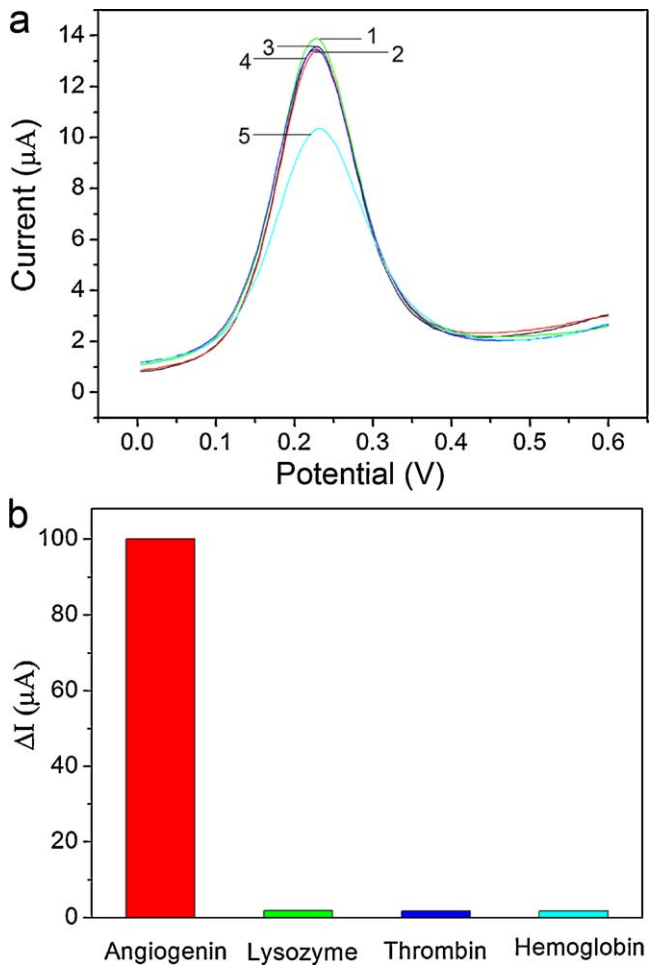


Fig. 3. (a) SWV for the anti-angiogenin aptamer/gold electrode after reaction with (1) 0.5 nM angiogenin, (2) 100 nM lysozyme, (3) 100 nM thrombin, (4) 100 nM hemoglobin, and (5) 1.0 nM angiogenin. (b) Relative response of the sensing system to the different proteins.

Table 2
Results of determination of Ang in serum samples.

Sample[a]	Clinical data (nmol L^{-1})	This method (nmol L^{-1})[b]	Percent of accuracy (%)
1	0.23	0.24	104
2	0.25	0.23	92
3	0.33	0.31	94
4	0.36	0.37	102

[a] The serum sample was diluted 50 times.
[b] The average of five determinations, detected with this method.

of accuracy of our proposed method was evaluated by comparison with the commercially used method. The percent of accuracy was calculated by the ratio between the concentration obtained by our method and the one obtained by the immunoassay. The accuracy between 92% and 104% (n = 5) shows a satisfactory result (Table 2). These results imply that the proposed angiogenin aptasensor has a promising feature for the analytical application in complex biological samples.

4. Conclusions

In this study, we propose a novel signal-off SWV electrochemical aptasensor for detection of angiogenin, in which an anti-angiogenin was used as a molecular recognition element, and $[Fe(CN)_6]^{4-/3-}$ as a redox probe. In order to select the best electrochemical method to do the target analysis, control experiments were carried out by SWV, CV and EIS. The results indicate that the SWV method is the most repeatable, sensitive and rapid way for angiogenin analysis. The proposed aptasensor shows a very wide linear detection range over 0.01–30 nM with a detection limit of 1 pM. In addition, it also exhibits remarkably high sensitivity, repeatability and selectivity. This protocol could potentially be applied to the aptamer-based detection of many other proteins and small molecules. Although, this approach has many desirable features, a few limitations need to be worked on in our future study. Such as, it is only an in vitro research, which is not suited for observing the overall effects of an experiment on a living subject. Another weakness of our method is the stability, because the major limitation of aptamers as molecular recognition elements is degradation by nucleases. However, from the analytical chemistry point of view, this new protocol is still promising for broad potential applications in clinic assay and protein analysis.

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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.2011.09.022.

References

Baker, B.R., Lai, R.Y., Wood, M.S., Doctor, E.H., Heeger, A.J., Plaxco, K.W., 2006. Am. Chem. Soc. 128, 3138–3139.
Bardea, A., Katz, E., Willner, I., 2000. Electroanalysis 12, 1097–1106.
Beutler, B., Cerami, A., 1986. Nature 320, 584–588.
Bicknell, R., Vallee, B.L., 1988. Proc. Natl. Acad. Sci. U. S. A. 85, 5961–5965.
Bicknell, R., Vallee, B.L., 1989. Proc. Natl. Acad. Sci. U. S. A. 86, 1573–1577.
Bogomolova, A., Komarova, E., Reber, K., Gerasimov, T., Yavuz, O., Bhatt, S., Aldissi, M., 2009. Anal. Chem. 81, 3944–3949.
Chang, H.X., Tang, L.H., Wang, Y., Jiang, J.H., Li, J.H., 2010. Anal. Chem. 82, 2341–2346.
Cho, H.S., Baker, B.R., Pagba, C.V., Laurence, T.A., Lane, S.M., Lee, L.P., 2008. Nano Lett. 8, 4386–4390.
Dijksma, M., Kamp, B., Hoogvliet, J.C., van Bennekom, W.P., 2001. Anal. Chem. 73, 901–907.
Ellington, A.D., Szostak, J.W., 1990. Nature 346, 818–822.
Fett, J.W., Strydom, D.J., Lob, R.R., Alderman, E.M., Bethune, J.L., Riordan, J.F., Vallee, B.L., 1985. Biochemistry 24, 5480–5486.
Frater-Schröder, M., Risau, W., Hallmann, R., Gautschi, P., Böhlen, P., 1987. Proc. Natl. Acad. Sci. U. S. A. 84, 5277–5281.
Jin, Y., Yao, X., Liu, Q., Li, J.H., 2007. Biosens. Bioelectron. 22, 1126–1130.
Katona, T.M., Neubauer, B.L., Iversen, P.W., Zhang, S.B., Baldridge, L.A., Cheng, L., 2005. Clin. Cancer Res. 11, 8358–8363.
Leibovich, S.J., Polverini, P.J., Shepard, H.M., Wiseman, D.M., Shively, V., Nuseir, N., 1987. Nature 329, 630–632.
Li, W., Wang, K.M., Tan, W.H., Ma, C.B., Yang, X.H., 2007. Analyst 132, 107–113.
Li, W., Yang, X.H., Wang, K.M., Tan, W.H., Li, H.M., Ma, C.B., 2008. Talanta 75, 770–774.
Minunni, M., Tombelli, S., Gullotto, A., Luzi, E., Mascini, M., 2004. Biosens. Bioelectron. 20, 1149–1156.
Nezamzadeh, A., Amini, M.K., Faghihian, H., 2007. Int. J. Electrochem. Sci. 2, 583–594.
Nobile, V., Russo, N., Hu, G.F., Riordan, J.F., 1998. Biochemistry 37, 6857–6863.
Okamura, K., Sato, Y., Matsuda, T., Hamanaka, R., Ono, M., Kohno, K., Kuwano, M., 1991. J. Biol. Chem. 266, 19162–19165.
Parham, H., Rahbar, N., 2010. J. Hazard Mater. 177, 1077–1084.
Ruan, C.M., Yang, L.J., Li, Y.B., 2002. Anal. Chem. 75, 4814–4820.
Shapiro, R., Riordan, J.F., Vallee, B.L., 1986. Biochemistry 25, 3527–3532.
St Clair, D.K., Ryback, S.M., Riordan, J.F., Vallee, B.L., 1987. Proc. Natl. Acad. Sci. U. S. A. 84, 8330–8334.
Tuerk, C., Gold, L., 1990. Science 249, 505–510.
Willner, I., Zayats, M., 2007. Angew. Chem. Int. Ed. 46, 6408–6418.
Zayats, M., Huang, Y., Gill, R., Ma, C.A., Willner, I., 2006. J. Am. Chem. Soc. 128, 13666–13667.
Zuo, X.L., Song, S.P., Zhang, J., Pan, D., Wang, L.H., Fan, C.H., 2007. Am. Chem. Soc. 129, 1042–1043.