Biosensors and Bioelectronics 86 (2016) 496–501

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Immobilization free electrochemical biosensor for folate receptor in cancer cells based on terminal protection

Jiancong Ni [a,b], Qingxiang Wang [b,*], Weiqiang Yang [a], Mengmeng Zhao [a], Ying Zhang [a], Longhua Guo [a], Bin Qiu [a], Zhenyu Lin [a,*], Huang-Hao Yang [a]

[a] Ministry of Education Key Laboratory of Analysis and Detection for Food Safety, Fujian Provincial Key Laboratory of Analysis and Detection for Food Safety, Department of Chemistry, Fuzhou University, Fuzhou 350116, China
[b] Department of Chemistry and Environment Science, Fujian Province University Key Laboratory of Analytical Science, Minnan Normal University, Zhangzhou 363000, China

ARTICLE INFO

Article history:
Received 9 May 2016
Received in revised form
2 July 2016
Accepted 6 July 2016
Available online 7 July 2016

Keywords:
Immobilization free
Electrochemical biosensor
Folate receptor
Cancer cells
Terminal protection

ABSTRACT

The determination of folate receptor (FR) that over expressed in vast quantity of cancerous cells frequently is significant for the clinical diagnosis and treatment of cancers. Many DNA-based electrochemical biosensors have been developed for FR detection with high selectivity and sensitivity, but most of them need complicated immobilization of DNA on the electrode surface firstly, which is tedious and therefore results in the poor reproducibility. In this study, a simple, sensitive, and selective electrochemical FR biosensor in cancer cells has been proposed, which combines the advantages of the convenient immobilization-free homogeneous indium tin oxide (ITO)-based electrochemical detection strategy and the high selectivity of the terminal protection of small molecule linked DNA. The small molecule of folic acid (FA) and an electroactive molecule of ferrocence (Fc) were tethered to 3′- and 5′-end of an arbitrary single-stranded DNA (ssDNA), respectively, forming the FA-ssDNA-Fc complex. In the absence of the target FR, the FA-ssDNA-Fc was degraded by exonuclease I (Exo I) from 3′-end and produced a free Fc, diffusing freely to the ITO electrode surface and resulting in strong electrochemical signal. When the target FR was present, the FA-ssDNA-Fc was bound to FR through specific interaction with FA anchored at the 3′-end, effectively protecting the ssDNA strand from hydrolysis by Exo I. The FR-FA-ssDNA-Fc could not diffuse easily to the negatively charged ITO electrode surface due to the electrostatic repulsion between the DNA strand and the negatively charged ITO electrode, so electrochemical signal reduced. The decreased electrochemical signal has a linear relationship with the logarithm of FR concentration in range of 10 fM to 10 nM with a detection limit of 3.8 fM (S/N=3). The proposed biosensor has been applied to detect FR in HeLa cancer cells, and the decreased electrochemical signal has a linear relationship with the logarithm of cell concentration ranging from 100–10000 cell/mL. Compared with the traditional heterogeneous electrochemical FR biosensors, the proposed biosensor owns the merits of the simplicity and high specificity, presenting the great potential application in the area of early diagnosis of cancers.

1. Introduction

Folate receptor (FR), cysteine-rich cell membrane glycoproteins, is commonly recognized as a magnetic tumor biomarker. It is highly expressed in various human malignant cancerous cells, like breast, ovarian, endometrial, brain, lung, prostate, kidneys, and so on (Henne et al., 2006; Kamaly et al., 2009; Lu et al., 2011). However, FR hardly exists in normal organs and tissues. Hence, the detection of FR can be used as a clinical diagnosis of aggressive or undifferentiated tumors at an advanced stage, which is accompanied with an increased density of FR (Li et al., 2014; He et al., 2016). Some typical methods have been extensively exploited for FR detection in the cancer diagnosis, for instance, radioimmunoassay (Antony et al., 1987), kinetic capillary electrophoresis (CE) (Petrov et al., 2005), fluorescence resonant energy transfer (FRET) (Clapp et al., 2004), surface plasmon resonance (SPR) (Cooper, 2002) *etc*. Although these methods have been identified as powerful approaches for the assays, they have some deficiencies such as tedious procedures, hazard to human health, time-consuming, sophisticated, and costly instruments.

It has been reported that FR can efficiently bind to the vitamin folic acid (FA) with high affinity (K_d~10^{-9} M) (Bharali et al., 2005),

* Corresponding authors.
E-mail addresses: axiang236@126.com (Q. Wang), zylin@fzu.edu.cn (Z. Lin).

http://dx.doi.org/10.1016/j.bios.2016.07.012
0956-5663/

and its conjugates can internalize into the cell through a receptor mediated endocytosis and they have been frequently employed in bioimaging and chemotherapeutic drug delivery (Pan et al., 2013; Wang et al., 2011). The single-stranded DNA (ssDNA) could be terminally linked a small molecule, FA. When FA was bound to its protein target FR, it could be protected from the degradation by 3′-termini specific exonuclease I (Exo I) (called terminal protection) (Wu et al., 2009). The reason lies in that a steric hindrance of the bound FR macromolecules prevents Exo I from approaching and cleaving the phosphodiester bond of ssDNA from 3′-termini. Based on this principle, many sensitive and selective biosensors have been subsequently developed for FR detection (Jiang et al., 2015; Wu et al., 2011; Yang and Gao, 2014; Zhu et al., 2015). The electrochemical strategy has attracted considerable attention due to its remarkable advantages, including low cost, high sensitivity, simplicity, and rapid response (Gao et al., 2016). Many investigations have been reported on electrochemical biosensors for FR based on terminal protection of small molecule-linked DNA. For example, a novel electrochemical FR biosensor was presented via the nicking endonuclease-assisted amplification strategy (Cao et al., 2012). A highly sensitive and specific electrochemical detection of FR was developed based on hybridization chain reaction-assisted formation of copper nanoparticles (Zhao et al., 2015). A sensitive and label-free electrochemical impedance biosensor was constructed as well (Wang et al., 2014). The above-mentioned biosensors show the character of the high specificity for FR detection, however all these sensors are heterogeneous assays that probes are immobilized on the surface of electrode firstly, which is laborious and time-consuming. Furthermore, for the FA modified probe, FR interactions and the enzyme catalysis occur at the interface between the solution and the electrode surface, so the efficiencies are relative row. Moreover, the electrode modification procedures might affect the reproducibility of the biosensors. Therefore, it is desirable to develop immobilization-free based electrochemical FR biosensors in which the interaction of the probe-FR occurs in homogeneous solution and hence overcomes the drawbacks in traditional immobilization electrochemical FR biosensors.

Indium tin oxide (ITO) electrode with negatively charged surface can repel DNA because the molecular skeleton of DNA contains the negatively charged phosphate. This character has been broadly applied to develop immobilization free electrochemical biosensors for different targets (Wei et al., 2014; Xuan et al., 2013). For example, an immobilization-free and enzyme-free electrochemical nucleic acid sensing strategy was designed in light of the electrostatic repulsion between ITO electrode and the negatively charged dendrimer of DNA/PNA (Xuan et al., 2015). Given this character and the exonuclease III-assisted autocatalytic target recycling strategy, an electrochemical DNA biosensor was proposed for target DNA and protein detection (Liu et al., 2014). A label-free and enzyme-free electrochemical microRNA biosensor was presented combining this character with hybridization chain reaction (Hou et al., 2015). Our group also constructed a series of sensitive electrochemical biosensors for DNA and protein (Tan et al., 2015a, 2015b).

To the best of our knowledge, no work has been reported, which couples the high specificity of terminal protection and ITO-based immobilization-free electrochemical FR biosensor. In this study, we constructed a homogeneous immobilization-free, facile, high sensitive, and selective electrochemical biosensor for the folate receptor detection, which combines the advantages of immobilization free and the terminal protection. The proposed biosensor has high specificity and can be used to identify the over-expressed FR from the HeLa cells with high efficiency.

2. Experimental section

2.1. Reagents

Ferrocene (Fc) modified oligonucleotides (ssDNA-Fc) were synthesized by Sangon Inc. (Shanghai, China), and the arbitrary sequence was 5′-Fc **ACCTGGGGGAGTATTGCGGAGGAAGGT** -NH_2-3′. Both folate receptor (FR) and folic acid (FA) were obtained from Sigma-aldrich (Shanghai, China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were provided by TCI (Shanghai, China). Exo I and the corresponding buffer were purchased from Thermo (Shanghai, China). Other chemicals were of analytical grade and used without further purification. Double-distilled water (Millipore, Direct-Q, 3resistance 18.2-MΩ) was used throughout the whole process. All DNA solutions were prepared in the phosphate-buffered saline (PBS, 100 mM, pH 7.4).

2.2. Preparation of folic acid linked ssDNA-Fc (FA-ssDNA-Fc)

Firstly, a ssDNA-Fc labeled with NH_2 at its 3′-end was tethered to FA via a conjugation by cross-linking agents (EDC and NHS) to form folic acid modified ssDNA-Fc (FA-ssDNA-Fc) (Wu et al., 2009). The solution was dialyzed against PBS (pH 7.4) with the 3 kD Amicon Ultra device to remove excess folic acid in process. Varying concentrations of FR was added to the FA-ssDNA-Fc solution to interact for 1 h in dark at 37 °C, and the macromolecule FR-FA-ssDNA-Fc was formed to protect the Exo I from digesting. The Exo I (100 U/mL) was added to the solution containing FA-ssDNA-Fc or FR-FA-ssDNA-Fc by incubation at 37 °C in dark for 1 h.

2.3. Electrochemical detection system

The different pulse voltammetry (DPV) was performed on a CHI 650D electrochemical analyzer (Chenhua Instruments, Shanghai, China) with a conventional three-electrode system, which consists of an ITO working, a platinum reference, and a platinum counter electrodes. The potential of the Pt reference electrode in the buffer was determined to be +0.36 V with respect to an Ag/AgCl reference electrode (Xuan et al., 2015). The ITO electrode was sonicated in a 10 g/L Alconox solution, propan-2-ol, and double-distilled water twice for 15 min in sequence before electrochemical detection, a negatively charged working electrode surface is obtained (Tan et al., 2015a,b).

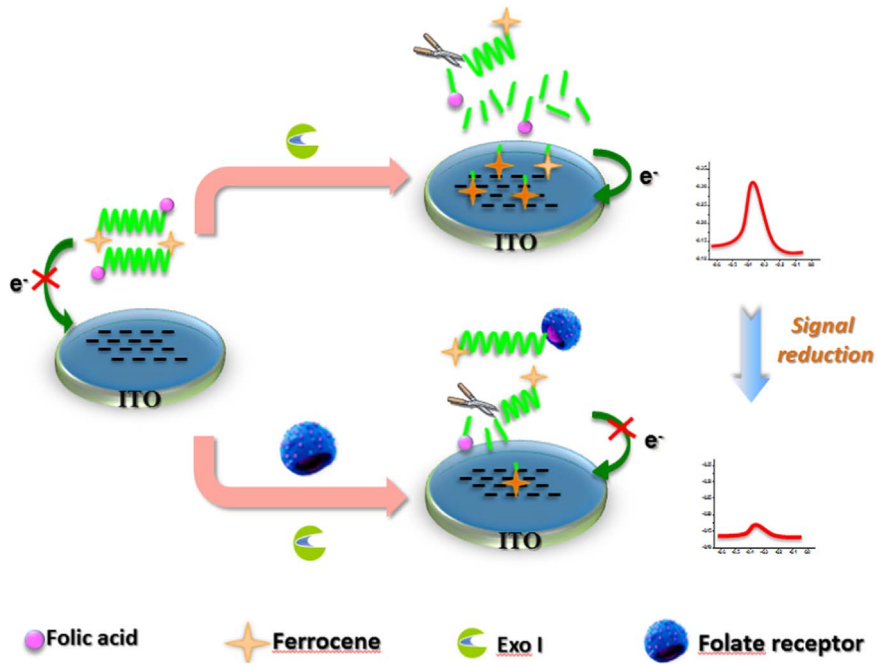
2.4. Cell culture

Human cervical cancer cells (HeLa cells) were cultured in RPMI 1640 medium with 5% CO_2 atmosphere at the condition of 37 °C, which was replenished with 10% fetal bovine serum (FBS) and 100 U/mL penicillin in an incubator. The cells were incubated by trypsin-EDTA for 1 min to prevent them from detaching from the flasks. After being washed with RPMI 1640 medium several times, the HeLa cells were centrifuged at 1000 rpm for 5 min at 4 °C and resuspended in a cold CHAPS lysis buffer (0.5% CHAPS, 1 mM $MgCl_2$, 0.1 mM PMSF, 1 mM EGTA, 10% glycerol, and 10 mM Tris-HCl (pH 7.4)). Then homogeneous cell lysate (10^5 cells/mL) was obtained in ice incubating. Ultimately, different concentrations of HeLa cells were diluted by 100 mM PBS (pH 7.4) for further use.

3. Results and discussion

3.1. Principle of the proposed biosensor for FR

Scheme 1 depicts the principle of the proposed immobilization



Scheme 1. The mechanism of electrochemical biosensor for FR determination based on the immobilization free and terminal protection of small molecule linked DNA.

free and terminal protection based electrochemical biosensor for FR. The approach realizes immobilization free by taking advantage of electrostatic repulsion between electroactive reporter-labeled ssDNA toward a negatively charged ITO electrode surface. The designed probe FA-ssDNA-Fc possesses a 27-mer ssDNA of arbitrary sequences, an electroactive ferrocence (Fc) tagged at the 5′-end, and a small molecule folic acid (FA) tagged at the 3′-end. The whole strain is negatively charged owing to the negatively charged phosphate on DNA molecular skeleton (Hu et al., 2014). The ITO working electrode surface can be negatively charged upon the special disposal (Xuan et al., 2013). The FA-ssDNA-Fc cannot diffuse freely to the ITO surface because of the electrostatic repulsion, and the DPV response is weak. The FA-ssDNA-Fc can be hydrolyzed into mononucleotides by Exo I from 3′-end without FR and therefore produces a free ferrocene molecule which will reach the ITO electrode surface and a strong DPV response appears. However, FR can bind with FA modified at the 3′-end of FA-ssDNA-Fc through high specificity of FR-FA interaction, preventing the FA-ssDNA-Fc from stepwise enzymolysis by the Exo I. Hence less free mononucleotide-Fc is produced and there is a small DPV signal. The decreased DPV signal has a relationship with the FR concentration, and an immobilization-free electrochemical biosensor for FR is established.

3.2. Feasibility of the proposed biosensor

Simple assays were performed to validate the proposed biosensor. As shown in Fig. 1, a strong DPV peak current at 0.032 V was observed in the solution containing FA-ssDNA-Fc and Exo I after incubation at 37 °C for 60 min (curve a). This probably because mononucleotide-Fc molecules are released from FA-ssDNA-Fc molecules after enzymolysis with Exo I, mononucleotide-Fc molecules reach the negative charged ITO electrode surface and result in strong DPV signals. When FR was added to the solution, the peak current decreased obviously (curve b). The reason lies in that the FR binds with FA which was labeled at 3′-end of ssDNA to initiate terminal protection reaction and less FA-ssDNA-Fc is hydrolyzed by Exo I. If the solution contained FA-ssDNA-Fc only,

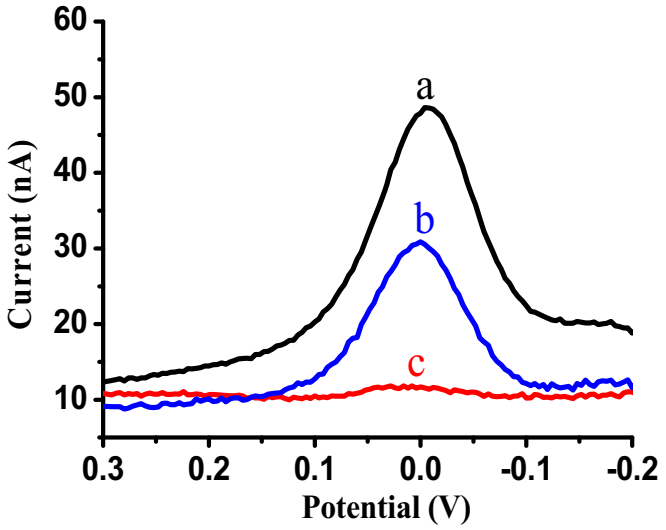


Fig. 1. DPV scans of reaction mixtures in the 100 mM PBS (pH 7.4) after incubation at 37 °C for 60 min at different conditions: a: 200 nM FA-ssDNA-Fc, 100 unit/mL Exo I; b: 200 nM FA-ssDNA-Fc, 500 pM FR and 100 unit/mL Exo I; c: 200 nM FA-ssDNA-Fc.

there was a very weak DPV signal (curve c). This is because it has strong electrostatic repulsion between the abundant negatively charged FA-ssDNA-Fc and the ITO electrode. These consequences clearly verify the feasibility of our assumption for the detection of FR.

3.3. Optimization of the reaction conditions

Some key factors which affect the performance of the biosensor were optimized. The interaction between the FA and FR plays an important role in this study, so the reaction time between FA-ssDNA-Fc and FR was firstly optimized. As shown in Fig. 2A, with the increase of the reaction time, the peak current of mononucleotide-Fc molecules that were degraded from the FA-ssDNA-Fc by Eox I decreased rapidly after the interaction of the FR and

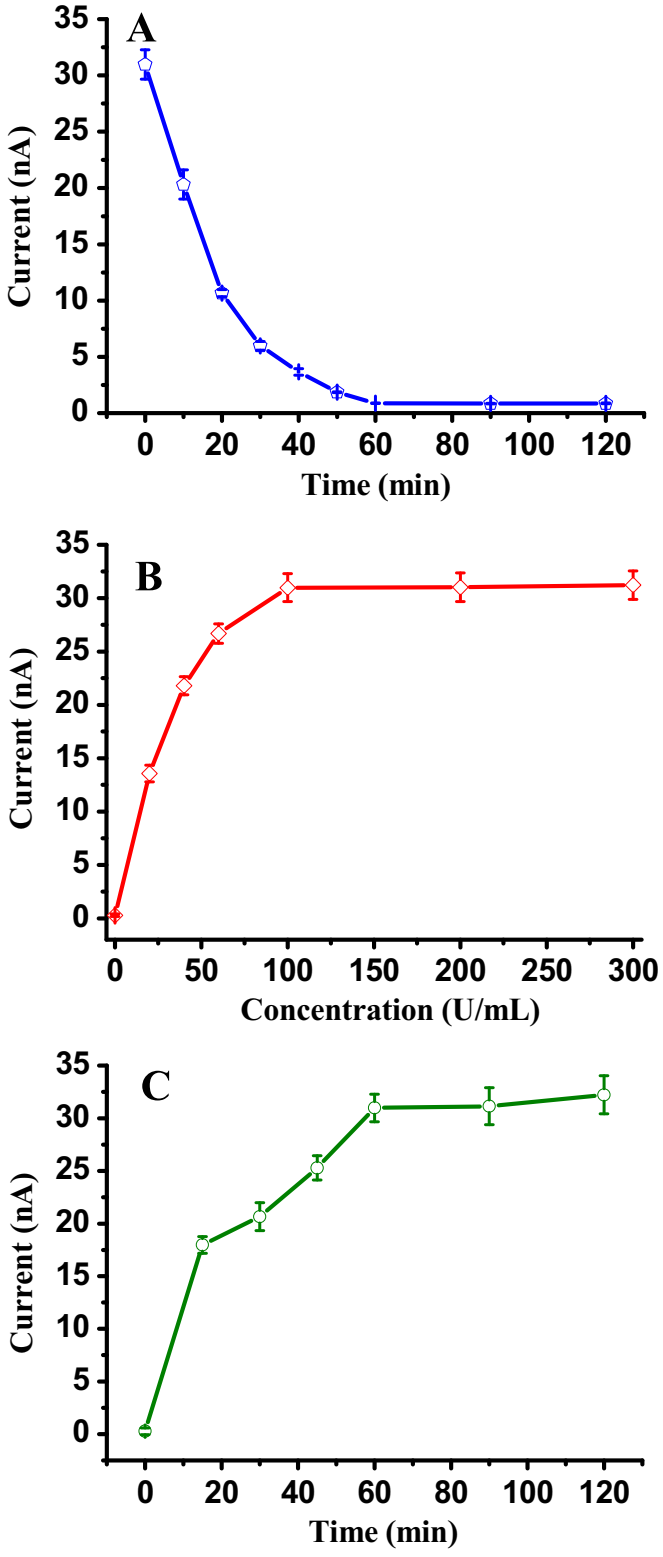


Fig. 2. Optimization of the reaction conditions in the 100 mM PBS (pH 7.4): (A) The effect of the FA-FR interaction time on DPV peak current. (B) The effect of the dosage of Exo I from 0 to 300 U/mL on the DPV peak current. (C) The effect of the time of Exo I enzymolysis on the DPV peak current. [FA-ssDNA-Fc]=200 nM, [FR]=200 nM, [Exo I]=100 unit/mL.

reached a constant value after 60 min. Thus, 60 min was employed as the optimum time for the FA-FR interaction. The concentration of Exo I was also investigated. Fig. 2B reveals that the peak current of mononucleotide-Fc molecules increased dramatically as the

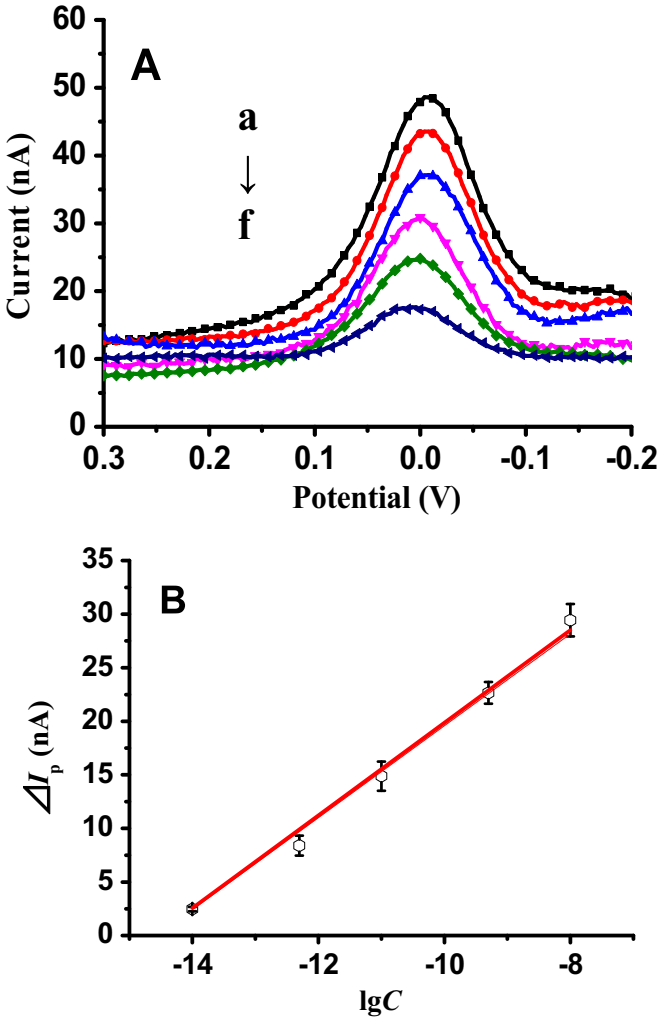


Fig. 3. (A) DPV scans in the presence of different concentrations of target FR in the 100 mM PBS (pH 7.4). (a) 0, (b) 10 fM, (c) 0.5 pM, (d) 10 pM, (e) 500 pM, (f) 10000 pM. (B) The relationship between the decrease of DPV peak current and the logarithm of FR concentration (lgC) in the 100 mM PBS (pH 7.4). [FA-ssDNA-Fc]=200 nM, [Exo I]=100 unit/mL.

dosages of Exo I increased from 0 to 300 U/mL and reached a plateau at 100 U/mL, suggesting that 100 U/mL of Exo I was enough to shear the FA-ssDNA-Fc (200 nM). The degraded time of the FA-ssDNA-Fc by Exo I was also optimized. As presented in Fig. 2C, the peak current of the mononucleotide-Fc molecules raised obviously with the extension of the reaction time by Exo I until it reached 60 min. Thus, 60 min was chosen as the degraded time of Exo I in the following study.

3.4. Calibration curve, reproducibility and stability of the biosensor

The sensitivity and dynamic range of the proposed electrochemical FR biosensor was evaluated by the addition of different concentrations of FR to the solution under the optimum reaction conditions. The DPV responses decreased with the increase of FR concentration (Fig. 3A). This phenomenon suggests that more FA-ssDNA-Fc are protected from the digestion through the FA binding with FR as more concentration of FR add. The decreased DPV peak current has a linear relationship with the logarithm FR concentration in the range of 10 fM to 10 nM (Fig. 3B). The regression equation is expressed as:

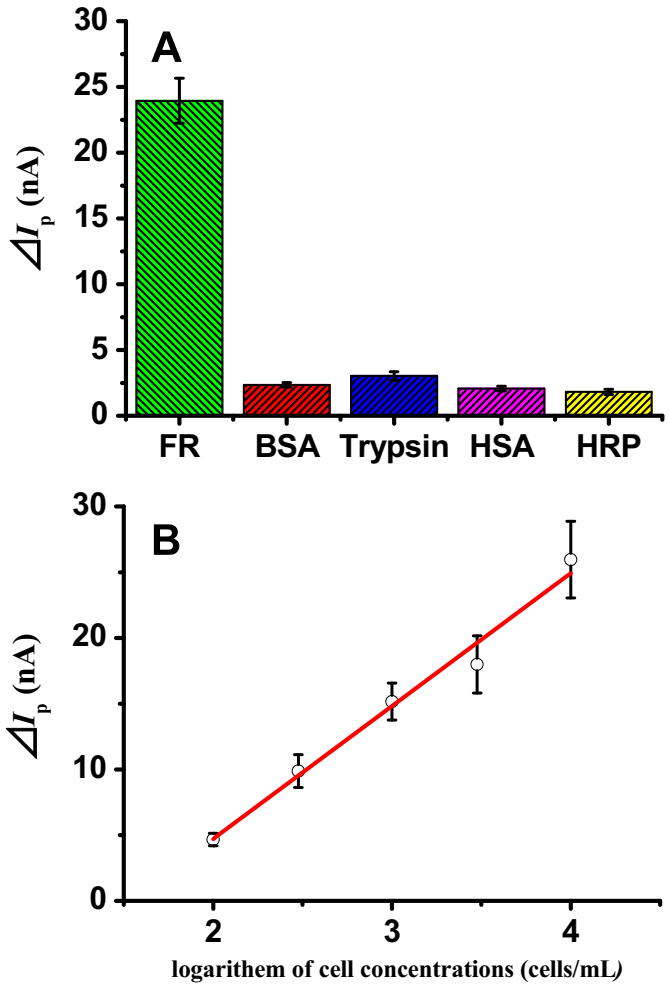


Fig. 4. (A) The selectivity of the proposed electrochemical biosensor in the 100 mM PBS (pH 7.4): (a) 1.0 nM FR, (b) 1.0 μM BSA, (c) 1.0 μM Trypsin, (d) 1.0 μM HSA, and (e) 1.0 μM HRP. (B) Determination of FR in the HeLa cell samples in the 100 mM PBS (pH 7.4): (a) 100 cells/mL, (b) 300 cells/mL, (c) 1000 cells/mL, (d) 3000 cells/mL, (e) 10000 cells/mL. The relationship between the decrease of DPV peak current and the logarithm of HeLa cell concentration. [FA-ssDNA-Fc]=200 nM, [Exo I]=100 unit/mL.

$$\Delta I_p = 4.291 \lg C + 62.49,\ R^2 = 0.9950$$

where ΔI_p is the difference of DPV peak current detected in the absence and presence of FR, C is the FR concentration, and R is the regression coefficient. The limit of detection (LOD) was estimated to be 3.8 fM based on the signal-to-noise characteristic (S/N=3).

Compared with other reported electrochemical FR biosensors without coupling with signal amplification strategies (Castillo et al., 2013; Li et al., 2014; Pan et al., 2013; Wang et al., 2014). The proposed biosensor has a wider linear range and lower detection limit. Furthermore, this biosensor is as good as some electrochemical sensors based on signal amplification (Cao et al., 2012; Wang et al., 2013a, b; Zhao et al., 2015).

The reproducibility and stability are two important properties of the proposed biosensor. Five parallel experiments at the concentration of 1.0 nM of FR were performed to examine the reproducibility of the proposed biosensor, the relative standard deviation (RSD) is only 5.5% (n=5), demonstrating that the proposed biosensor has good reproducibility. The stability of the proposed biosensor was further studied in the same solution containing FR (1.0 nM) through 8 times repeatedly detection using the same ITO electrode. The RSD is as low as 3.6% (n=8), which shows that the biosensor exhibits high stability.

3.5. Interference study and determination of HeLa cells

To verify the selectivity of the proposed sensor, 10 μM bovine serum albumin (BSA), trypsin, albumin human (HSA), horseradish peroxidase (HRP) were chosen as interferents. The concentration of FR was set as only 1.0 nM (1/1000-fold dosage of interferents). Fig. 4A shows that the values of ΔI_p from above-mentioned interferents were low whereas the values of ΔI_p were high in the presence of FR. This is because strong and specific FA-FR interaction can prevent FA-ssDNA-Fc from enzymolysis by Exo I. Hence, the proposed electrochemical biosensor has high selectivity.The developed biosensor was eventually applied to measure the cell concentration of HeLa cells containing over-express FR. The decreased DPV peak current has a linear relationship with the logarithm of HeLa cell concentration in the range of 100–10000 cell/mL (Fig. 4B). The regression equation can be expressed as:

$$\Delta I_p = 10.11 \lg C - 15.52,\ R^2 = 0.9914$$

where ΔI_p is the difference of DPV peak current of the biosensor in the absence and presence of HeLa cells, C is the HeLa cells concentration, and R is the correlation coefficient. The LOD was estimated to be 50 cell/mL (S/N=3). These results indicate that the proposed biosensor can be successfully applied to detect HeLa cells with high sensitivity.

More importantly, the developed biosensor has wider linear range and lower detection limit, and is selective toward FR-rich HeLa cells when compared with other early reported electrochemical HeLa cell biosensors regarding FR overexpression (Castillo et al., 2013; Jiang et al., 2014; Wang et al., 2013a, 2013b; Zheng et al., 2012),

4. Conclusion

In summary, we have demonstrated a simple, selective, and sensitive electrochemical biosensor to determine FR. The biosensor combines the merits of immobilization free and terminal protection of small molecule linked DNA, exhibiting a wide linear range and a low LOD. Although FR is over-expressed in the vast majority of cancers, yet it is low in the normal tissues. Thus the proposed biosensor can act as an available assay tool for FR detection in the early diagnosis of cancers. Furthermore, this proposed biosensor has great potential to become a feasible detection of different targets using small molecule–protein interaction strategy.

Acknowledgments

This work was financially supported NSFC for Excellent Youth Scholars of China (No. 21222506), NSFC (Nos. 21175024, 21275127 and 21275031), Nature Sciences Funding of Fujian Province (No. 2014J06005), Program for New Century Excellent Talents in University (NCET-12-0619) and the Program for Changjiang Scholars and Innovative Research Team in University (No. IRT15R11).

References

Antony, A.C., Verma, R.S., Kincade, R.S., 1987. Anal. Biochem. 162, 224–235.
Bharali, D.J., Lucey, D.W., Jayakumar, H., Pudavar, H.E., Prasad, P.N., 2005. J. Am. Chem. Soc. 127, 11364–11371.
Cao, Y., Zhu, S., Yu, J., Zhu, X., Yin, Y., Li, G., 2012. Anal. Chem. 84, 4314–4320.
Castillo, J.J., Svendsen, W.E., Rozlosnik, N., Escobar, P., Martinez, F., Castillo-Leon, J., 2013. Analyst 138, 1026–1031.
Clapp, A.R., Medintz, I.L., Mauro, J.M., Fisher, B.R., Bawendi, M.G., Mattoussi, H.,

2004. J. Am. Chem. Soc. 126, 301–310.
Cooper, M.A., 2002. Nat. Rev. Drug. Discov. 1, 515–528.
Gao, F., Gao, C., He, S., Wang, Q., Wu, A., 2016. Biosens. Bioelectron. 81, 15–22.
He, L., Wang, Q., Mandler, D., Li, M., Boukherroub, R., Szunerits, S., 2016. Biosens. Bioelectron. 75, 389–395.
Henne, W.A., Doorneweerd, D.D., Lee, J., Low, P.S., Savran, C., 2006. Anal. Chem. 78, 4880–4884.
Hou, T., Li, W., Liu, X., Li, F., 2015. Anal. Chem. 87, 11368–11374.
Hu, Y., Xu, X., Liu, Q., Wang, L., Lin, Z., Chen, G., 2014. Anal. Chem. 86, 8785–8790.
Jiang, H., Wang, X., 2014. Anal. Chem. 86, 6872–6878.
Jiang, H., Xu, G., Sun, Y., Zheng, W., Zhu, X., Wang, B., Zhang, X., Wang, G., 2015. Chem. Commun. 51, 11810–11813.
Kamaly, N., Kalber, T., Thanou, M., Bell, J.D., Miller, A.D., 2009. Bioconj. Chem. 20, 648–655.
Li, R., Wang, C., Hu, Y., Zheng, O., Guo, L., Lin, Z., Qiu, B., Chen, G., 2014. Biosens. Bioelectron. 58, 226–231.
Liu, S., Lin, Y., Wang, L., Liu, T., Cheng, C., Wei, W., Tang, B., 2014. Anal. Chem. 86, 4008–4015.
Lu, J., Pang, Y., Xie, F., Guo, H., Li, Y., Yang, Z., Wang, X., 2011. Nucl. Med. Biol. 38, 557–565.
Pan, W., Yang, H., Zhang, T., Li, Y., Li, N., Tang, B., 2013. Anal. Chem. 85, 6930–6935.
Petrov, A., Okhonin, V., Berezovski, M., Krylov, S.N., 2005. J. Am. Chem. Soc. 127, 17104–17110.
Tan, Y., Wei, X., Zhang, Y., Wang, P., Qiu, B., Guo, L., Lin, Z., Yang, H.H., 2015a. Anal. Chem. 87, 11826–11831.
Tan, Y., Wei, X., Zhao, M., Qiu, B., Guo, L., Lin, Z., Yang, H.H., 2015b. Anal. Chem. 87, 9204–9208.
Wang, G., He, X., Wang, L., Zhang, X., 2013a. Biosens. Bioelectron. 42, 337–341.
Wang, H.B., Zhang, H.D., Xu, S.P., Gan, T., Huang, K.J., Liu, Y.M., 2014. Sens. Actuators B 194, 478–483.
Wang, X., Li, J., Wang, Y., Koenig, L., Gjyrezi, A., Giannakakou, P., Shin, E.H., Tighiouart, M., Chen, Z., Nie, S., 2011. ACS Nano 5, 6184–6194.
Wang, Z., Chen, S., Hu, C., Cui, D., Jia, N., 2013b. Electrochem. Commun. 29, 4–7.
Wei, X., Ma, X., Sun, J.J., Lin, Z., Guo, L., Qiu, B., Chen, G., 2014. Anal. Chem. 86, 3563–3567.
Wu, Z., Wang, H., Guo, M., Tang, L.J., Yu, R.Q., Jiang, J.H., 2011. Anal. Chem. 83, 3104–3111.
Wu, Z., Zhen, Z., Jiang, J.H., Shen, G.L., Yu, R.Q., 2009. J. Am. Chem. Soc. 131, 12325–12332.
Xuan, F., Fan, T.W., Hsing, I.M., 2015. ACS Nano 9, 5027–5033.
Xuan, F., Luo, X., Hsing, I.M., 2013. Anal. Chem. 85, 4586–4593.
Yang, X., Gao, Z., 2014. Nanoscale 6, 3055–3058.
Zhao, J., Hu, S., Cao, Y., Zhang, B., Li, G., 2015. Biosens. Bioelectron. 66, 327–331.
Zheng, T.T., Zhang, R., Zou, L., Zhu, J.J., 2012. Analyst 137, 1316–1318.
Zhu, Y., Wang, G., Sha, L., Qiu, Y., Jiang, H., Zhang, X., 2015. Analyst 140, 1260–1264.