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An electrochemical label-free and sensitive thrombin aptasensor based on graphene oxide modified pencil graphite electrode

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ABSTRACT

In this work, we tactfully constructed a novel label-free electrochemical aptasensor for rapid and facile detection of thrombin using graphene oxide (GO) and thrombin binding aptamer (TBA).

The strategy relies on the preferential adsorption of single-stranded DNA (ssDNA) to GO over aptamer-target complexes. The TBA-thrombin complex formation was monitored by differential pulse voltammetry (DPV) using the guanine oxidation signal. In the absence of thrombin, the aptamers adsorbed onto the surface of GO leading to a strong background guanine oxidation signal. Conversely, in the presence of thrombin, the conformational transformation of TBA after incubating with the thrombin solution and formation of the aptamer-thrombin complexes which had weak binding ability to GO, leads to the desorption of TBA-thrombin complex from electrode surface and significant oxidation signal decrease.

The selectivity of the biosensor was studied using other biological substances. The biosensor's signal was proportional to the thrombin concentration from 0.1 to 10 nM with a detection limit of 0.07 nM.

Particularly, the proposed method could be widely applied to the aptamer-based determination of other target analytes.

1. Introduction

Quantitative detection of proteins have attracted great attention in biomedical fields, including disease diagnosis and basic discovery research (Rosi and Mirkin, 2005; Wu et al., 2007). Thrombin is a kind of serine protease with variable concentration in blood. Thrombin not present in blood under normal conditions, can reach low-micromolar concentrations when blood coagulation occurs. Low levels (low nM) of thrombin generated early in hemostasis (Shuman and Majerus, 1976) and high-picomolar range in blood of patients suffering from diseases known to be associated with coagulation abnormalities (Bichler et al., 1996). Moreover, concentration of thrombin is crucial in other pathological conditions including the central nervous system injury, thromboembolic disease, Alzheimer's disease and tissue repair at the vessel wall (Francois and David, 1954; Nishino et al., 1993). Therefore, highly sensitive and selective detection of thrombin in blood is of great interest and importance for both research and clinical diagnosis applications (Holland et al., 2000). Aptamers are usually artificial DNA or RNA can be selected using the SELEX procedure (systematic enrichment of ligands by exponential amplification) from a library of nucleic acids containing $\sim 10^{15}$ individual sequences. Aptamers bind specifically to the molecular or biomolecular entities with high affinity and thus often inhibit target-associated biological functions (Zhang et al., 2007; Ellington and Szostak, 1990; Huang and Zhu, 2009).

Compared with the traditional recognition element antibody, aptamers are much easier for synthesis and preservation and have a number of unique features that make them a more effective choice than antibodies, including low cost, large-scale production, increased stability against high temperature and extreme pH, long half-life, and reversible denaturation (Zhang et al., 2010). Therefore, aptamer has been increasingly used as an attractive recognition element for the development of new biosensor which is named as aptasensor (Zhao et al., 2012; Chen et al., 2012; Bang et al., 2013).

Numerous strategies and technologies of aptamer based protein detections have been developed, such as fluorescence (Pavlov et al., 2005; Wang et al., 2005) colorimetric (Huang et al., 2005), chemiluminescent (Yan et al., 2009; Song et al., 2014), electrochemiluminescent (Zhuo et al., 2015), quartz crystal microbalance (Liss et al., 2002), surface plasmon resonance (SPR) (Lee et al., 2008; Wang et al., 2009), electrochemical detection (Xu et al., 2005; Ikebukuro et al., 2005; Zhao et al., 2015) and so on. Among these methods, the electrochemical detection have attracted particular attention in the development of aptasensors because of low

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background, simple operation, rapid response, high sensitivity, miniaturization, low cost, and etc. (Qin et al., 2012; Wu et al., 2013; Chen et al., 2011; Zhang et al., 2007).

Electrochemical aptasensors are classified into major two label-free and label based protocols. label-free monitoring seems to be a simple, less time consuming and more applicable strategy in comparison with the others.

Graphene (GN) and its derivative GO, a new kind of carbon materials that consists of only one plain layer of atoms arranged in a honeycomb lattice, exhibits a number of interesting properties, such as unique electrical conductivity, high specific surface area, low manufacturing cost and easy functionalization (Cote et al., 2009; Geim and Novoselov, 2007). GO has superior binding to ssDNA over rigid dsDNA or aptamer-target complexes and has been used for the production of biosensors for detecting nucleic acids (Guo et al., 2013; Song et al., 2013), proteins (Yuan et al., 2013; Sharon et al., 2013), and small molecules (He et al., 2011; Zhu et al., 2012).

Herein, we developed an electrochemical biosensor for the determination of thrombin based on decrease of guanine oxidation signal after interaction between nucleotides and thrombin.

As shown in Scheme 1, the pencil graphite electrode (PGE) modified by GO formed a platform for the adsorption of the TBA; then the biosensing electrode was immersed into a certain concentration of thrombin solution for a period of time; after binding with the thrombin, the conformational transition of TBA leads to the desorption from the surface of GO and decreasing of guanine oxidation signal. This strategy was demonstrated as a convenient, sensitive and selective detection platform for a range of target analytes.

2. Experimental

2.1. Materials

The pencil graphite was obtained as pencil lead from Rotring Co. LTD, Germany (R 505210 N) of type H. All leads had a diameter of 2.0 mm and were used as received. Oligonucleotides were purchased as lyophilized powder from MWG-Biotech company, with the following sequence: ss-TBA: 5′-GGT TGG TGT GGT TGG-3′; and a random DNA oligonucleotide 5′-GCG GCG CCG CTG CGG.

The stock solutions of the oligonucleotides (500 μg/mL) were prepared with TE buffer solution (10 mM Tris–HCl, 1 mM EDTA, pH 7.00) and kept frozen. More diluted solutions of the oligonucleotides were prepared by diluting stock solution with phosphate buffer solution pH 5 (PBS, 0.1 M NaH_2PO_4/Na_2HPO_4, 0.10 M NaCl, pH 5). All chemicals were of analytical reagent grade. Distilled, deionized and sterilized water was used in all solution preparation. All DNA solutions were kept frozen at −20 °C and all the experiments were performed at room temperature in an electrochemical cell.

2.2. Apparatus

Electrochemical experiments were performed using AUTOLAB PGSTAT 30 electrochemical analysis system and GPES 4.7 software package (Eco Chemie. The Netherlands). The utilized three-electrode system was composed of a PGE (surface area of 0.0314 cm^2) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum wire as the auxiliary electrode.

2.3. Synthesis of graphene oxide

Graphene oxide (GO) was synthesized directly from graphite by a modified Hummers method (Cote et al., 2009; William et al., 1958). Generally, 1 g graphite was ground with 50 g NaCl for 10 min. NaCl was then dissolved and removed by filtration with water. The remaining graphite was stirred in 23 mL of 98% H_2SO_4 for 8 h. $KMnO_4$ (3 g) was gradually added while keeping the temperature less than 20 °C. The mixture was then stirred at 80 °C for 45 min. Next, water (46 mL) was added and the mixture was heated at 105 °C for 30 min. The reaction was terminated by addition of distilled water (140 mL) and 30% H_2O_2 solution (10 mL). The resulting mixture was washed by repeated centrifugation and filtration, first with 5% HCl aqueous solution and then distilled water. Finally, the graphene oxide (GO) product was obtained after dried in vacuum.

2.4. Procedure

2.4.1. Preparation of the working electrode

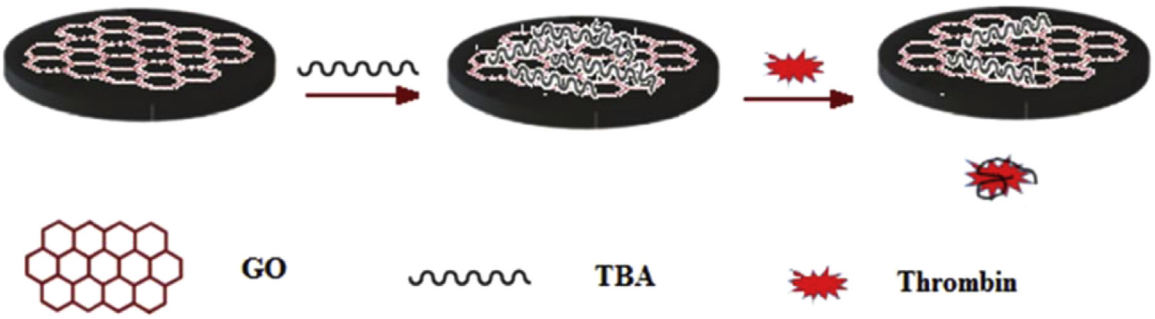
The body of pencil lead was tightly coated with Teflon band. Electrical contact with the lead was achieved by soldering a copper wire to the metallic holder of the working electrode. The pencil lead was fixed vertically and immersed in the solution in which the contact was only achieved via cross section of the electrode. The surface was polished on a weighing paper to a smoothed finish then sonicated in nitric acid and doubly distilled water in turn. 1.0 mg graphene oxide was dispersed in 1 mL H_2O to form a homogenous mixture. 3 μL of 1.0 mg mL^{-1} mixtures were dropped on the surface of PGE and dried at room temperature before each use. The prepared electrode can be storage for at least one month at room temperature which is usable in this duration.

2.4.2. TBA immobilization

The modified electrode was immersed into the TBA solution in order to be adsorbed on the surface of GO/PGE. The adsorption process was kept for at least 20 min at room temperature followed by being thoroughly rinsed in a stirred PBS pH 5 before each experiment.

2.4.3. Aptamer-target interaction

Aptamer-target interaction was performed by dipping the



Scheme1. Schematic illustration of the label-free electrochemical aptasensor for thrombin detection.

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aptamer-modified electrode into 1 mL PBS (pH 7.00) containing certain concentration of thrombin for 15 min at room temperature. Then, the electrode was rinsed in a stirred PBS solution for optimum time to remove any unbound or weakly bound substances.

2.4.4. Voltammetric measurements of thrombin

The electrochemical behavior of the electrode surface was studied using anodic differential pulse voltammetry (ADPV) in 20 mM PBS solution (pH 7.00) and scanning the electrode potential between 0.50 and 1.2 V at pulse amplitude of 25 mV.

The raw data were treated using the Savitzky and Golay filter (level 2) of the GPES software, followed by the GPES software moving average baseline correction using a 'peak width' of 0.01. Repetitive measurements were carried out following renewing the PGE surface by cutting and polishing of the electrode. Each experiment repeated at least 3 times.

3. Results and discussion

3.1. Preliminary investigation

The morphology of the Go/PGE was characterized using SEM. The SEM image is shown in Fig. S1A. It can be observed that the prepared graphene oxide illustrates the flake-like shape and layer–layer structure of graphene oxide edges.

In order to investigate the electrochemical behavior of TBA on the bare, activated and GO modified PGE, aptamer DNA is immobilized on the surface of three different electrode by simple immersing method for 30 min. Fig. S1B shows the DPVs obtained in 20 mM PBS solution (pH 7.00) for bare PGE (curve a), activated PGE (curve b), GO/PGE (curve c), TBA immobilized on the untreated PGE (curve d), activated PGE (curve e) and GO/PGE (curve f). As seen in Fig. S1B, the guanine oxidation signal appears in the presence of TBA immobilized electrodes at about 0.95 V, and the peak height for GO modified PGE is about 3 times higher than that of activated PGE. These results is probably related to the unique properties of GO such as high surface area and rapid electron transport capability leads to the higher current value in comparison with unmodified PGE. Then, we studied TBA immobilization condition on the GO modified PGE surface.

3.2. Optimization of aptamer immobilization conditions

In order to investigate the electrochemical behavior of TBA on the GO/PGE, Different amounts of TBA were immobilized on the GO modified PGE. As shown in Fig. S2A, the variation is linear up to about 6 μM which revealed the increased levels of DNA aptamer adsorbed onto the GO surface and then levels off for greater concentrations may be due to full coverage of the electrode surface. Therefore 6 μM is suggested as a suitable concentration for TBA immobilization at electrode surface. The effect of aptamer immobilization time on the GO modified PGE was also investigated. The results obtained from the voltammetric measurements revealed that the guanine oxidation signal elevated as the immobilization time increased to about 20 min and remained constant between 20 and 60 min (Fig. S2B). Therefore 20 min is suggested as a suitable time for TBA immobilization at electrode surface.

3.3. Possibility for label-free electrochemical detection of thrombin

Detection of the hybridization was accomplished by monitoring difference between the guanine oxidation signal of TBA modified GO/PGE before and after interaction with thrombin. As shown in Fig. 1, in the absence of thrombin, the aptamers adsorbed onto the surface of GO/PGE leading to a strong background DPV signal. Conversely, in the presence of thrombin, the conformational transition of TBA after interaction by thrombin leads to desorption of aptamer-thrombin complexes form GO/PGE resulting in a significant DPV signal decrease. Having observed the ability of the electrode in detection of thrombin, a random DNA oligonucleotide with the same concentration as TBA, was used as negative control. Results showed that in terms of random DNA oligonucleotide, DPV signals maintain almost the same both in the absence and presence of thrombin.

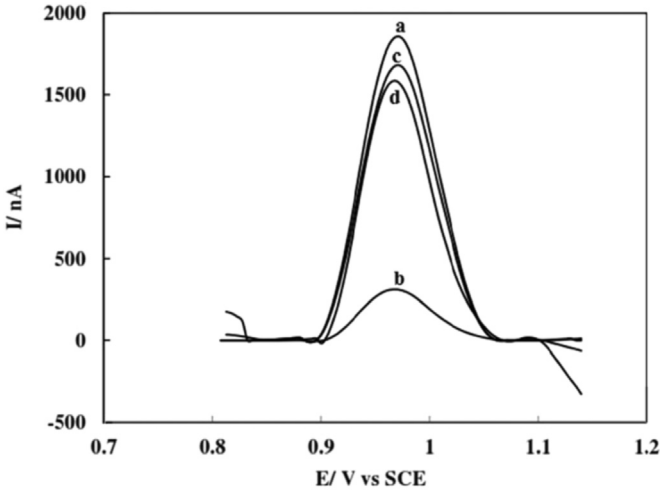

Fig. 1. DPV response of TBA/GO/PGE before (a) and after (b) interaction with 20 nM thrombin and random DNA/GO/PGE before (c) and after (d) interaction with 20 nM thrombin. Electrode conditions: immersion in 6 μM TBA or 6 μM random DNA solution for 20 min; incubation time: 30 min. Other experimental and voltammetric conditions were as described in Section 2.4.

3.4. Optimization of the assay conditions

In order to obtain the optimal biosensor operating conditions, some experimental variables such as pH value and reaction time affecting the sensing performance were studied, and special emphasis was given to the optimization of such experimental variables.

One of the important factors which can affect the formation of thrombin-aptamer complexes and the ssDNA adsorption onto GO/PGE is pH. Fig. 2A represents a histogram illustrating the guanine oxidation signal of aptamer adsorbed on GO/PGE (a) and after interaction with thrombin (b) at different pH values. On the basis of obtained results, pH 5 selected for TBA immobilization that shows by raising pH, the electrostatic repulsion between DNA and GO increases; and pH 7 for thrombin and DNA interaction probably due to the fact that the best interaction between TBA and thrombin occurs under the neutral condition. Therefore, pH 7 was selected as the optimal pH value for this step of experiments.

The influence of the incubation time of TBA/GO/PGE and thrombin on the performance of the biosensing electrode at thrombin concentration of 10 nM was also investigated. As shown in Fig. 2B, by increasing the incubation time up to 15 min, the difference between guanine oxidation signal of TBA/GO/PGE before and after interaction with thrombin increased and then leveled off after 15 min Therefore, 15 min was chosen for further experiments.

We investigated the effect of electrode washing on the removal of any unbound or weakly bound substances from the electrode surface before each measurement. As shown in Fig. 2C, at the first seconds of electrode washing, guanine oxidation signal decreased slightly, and then remained unchanged for longer washing times.

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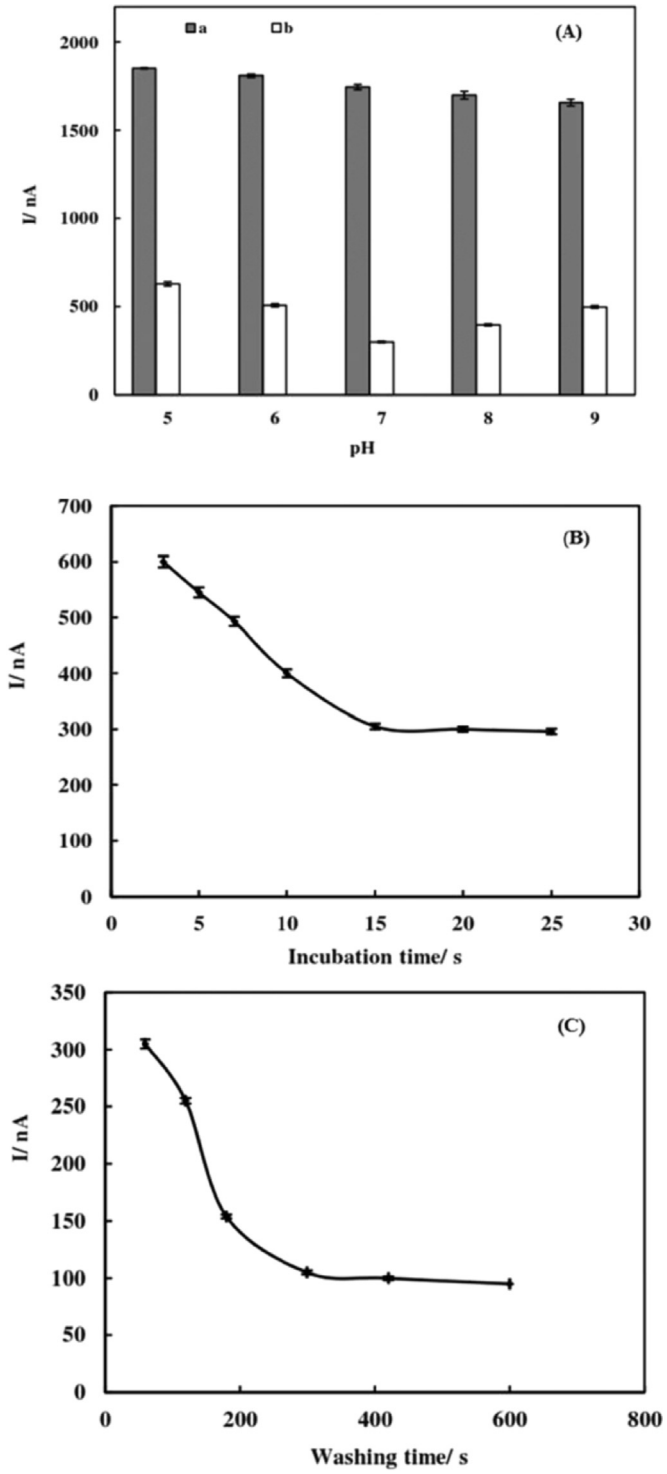

Fig. 2. Optimization of TBA and Thrombin interaction condition (A) The effect of pH of aptamer immobilization solution and incubation solution on the obtained guanine oxidation signal; (B) The effect of incubation time on DPV response; (C) The effect of washing time of the TBA/GO/PGE after interaction with thrombin at concentration of 10 nM. Experimental and voltammetric conditions were as described in Fig. 1.

Therefore, it was concluded that at the experimental conditions, washing of the electrode up to 300 s can eliminate the unwanted substances from electrode surface. Therefore we selected 300 s as washing time before each measurement.

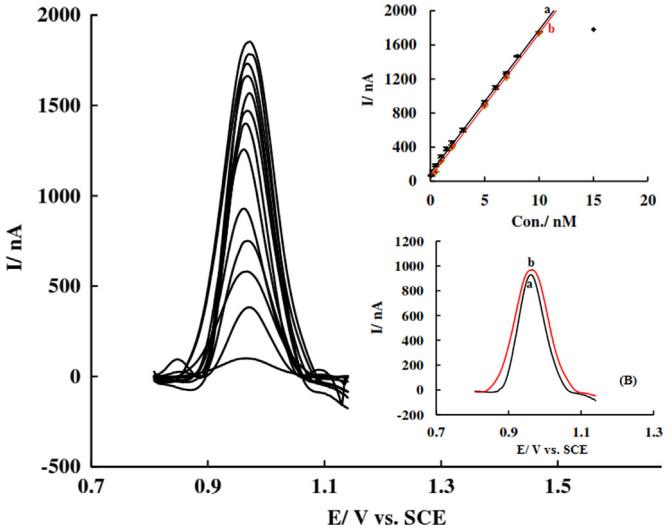

Fig. 3. DPV responses of the TBA/GO/PGE after interaction with different concentration of thrombin; Inset A: the calibration graph of the difference between the oxidation signals of the aptamer modified GO/PGE in the presence and absence of thrombin in PBS (curve a) and in diluted serum sample (curve b) as a function of the concentration of thrombin; Inset B: Typical DPV response of 5nM thrombin in PBS (curve a) and diluted serum sample (curve b). Electrode conditions: immersion in 6μM TBA for 20min; incubation time: 15min; washing time: 300s.

3.5. Reproducibility and stability of the biosensor

The reproducibility of the biosensor was evaluated by analysis of the same concentration of thrombin (2 nM) using five biosensors under the same conditions. All biosensors exhibited close DPV signal, and a relative standard deviation (RSD) of 1.68% was obtained, which indicated that the reproducibility of the proposed biosensor is acceptable. When the aptamer-modified electrode was stored in PBS (pH 7) at 4 °C, it retained 92% of its initial current after one week storage which displays good stability of the biosensor.

3.6. Sensitivity and selectivity of the biosensor

Fig. 3 shows the DPV response of TBA/GO/PGE before and after interaction with various concentrations of thrombin. Under the optimized test conditions, the sensitivity of the proposed biosensor were assessed by measuring the difference between the guanine oxidation signals of the aptamer modified GO/PGE in the presence and absence of thrombin (ΔI) upon the concentration of thrombin. As shown in inset A Fig. 3, ΔI was enhanced when a higher concentration of thrombin was used for binding with the TBA and leveled off at ca. 15nM thrombin. Thus, at this concentration of thrombin, the maximum capacity of the probe available on the electrode surface is leached from electrode surface. As seen in inset A of Fig. 3, the calibration graph is linear between 0.1 and 10nM with correlation coefficient of 0.998 (curve a). The detection limit calculated by means of equation: y LOD (ΔI)=yB+3Sy/x and regression equation: y (ΔI)=167.65x(μM)+97.835 was about 0.07nM under optimal condition. Such detection limit compares favorably with those reported for the other thrombin biosensors shown in Table 1.

The selectivity of the biosensor was examined by incubating the biosensor in the aqueous solutions containing thrombin at the concentration of 10 nM, while containing HSA, BSA, tyrosine, phenyl alanine, and hemoglobin in the same test condition at the concentration of 40 nM. The selectivity of the biosensor was also accomplished in samples containing both thrombin and other interfering species. As shown in Fig. 4, interaction between TBA/GO/

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Table 1
Performance comparison of different aptasensors.

Detection technology	Linear range (μM)	Detection limit	Reference
Fluorescence	0.5–20nM	0.18nM	Wang et al., 2011
Chemiluminessence	2–80nM	1.4nM	Song et al., 2014
Electrochemiluminescence	0.5–25nM	0.21nM	Zhuo et al., 2015
Electrochemical impedance spectroscopy	0.12–30nM	0.03nM	Li et al., 2008
Differential pulse voltammetry	1–60nM	0.5nM	Kang et al., 2008
Differential pulse voltammetry	6–60nM	3nM	Yan et al., 2011
Differential pulse voltammetry	5–35nM	0.5nM	Radi et al., 2006
Differential pulse voltammetry	0.1–10nM	0.07nM	This work

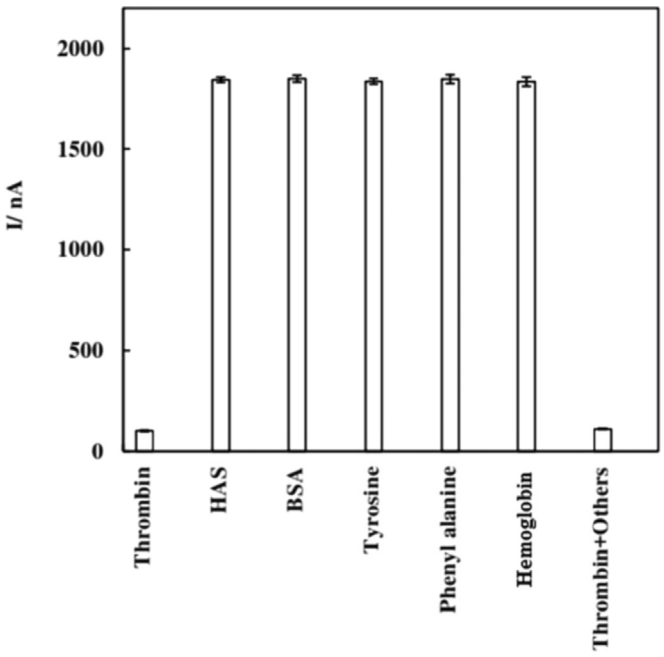

Fig. 4. Selectivity of the biosensor to thrombin compared to HSA, BSA, tyrosine, phenyl alanine, and hemoglobin as competitive species; Concentration of thrombin is 10 nM and the others are 40 nM. Experimental and voltammetric conditions were as described in Fig. 3.

PGE and these species did not change the guanine oxidation signal in selected experimental conditions due to the absence of effective interaction between TBA and these species. On the other hand, presence of interfering species in the mixture solutions has negligible effect on the interaction between immobilized aptamer and target suggesting that the proposed biosensor possessed an excellent selective response to thrombin, which was attributed to the high selectivity of aptamer to its target.

3.7. Detection of thrombin in human serum samples

In order to evaluate the analytical reliability and application potential of this biosensor in clinical analysis, the proposed biosensor was used for detection of thrombin in healthy human blood serum samples under optimal experimental conditions. We performed experiments with 100-fold diluted human blood serum samples; a typical DPV signal of thrombin under controlled condition and in serum has been shown in the inset (B) of Fig. 3. These results show that similar responses were found for both buffer and serum samples, and prove applicability of the proposed sensor for real sample analysis. Moreover, we used this prepared biosensor for determination of thrombin concentration in spiked diluted serum sample by the standard addition method. The obtained results for measurements at four different concentrations (Table S1) showed good recovery values (98–103%) and the relative standard deviation is between 0.82% and 2.44%. The results indicated that our proposed biosensor has high accuracy in practical applications and can be used for the thrombin detection in real biological samples.

4. Conclusions

In conclusion, we have developed a novel label-free electrochemical aptasensing platform based on graphene oxide nanosheets adsorbed aptamer for simple and rapid determination of thrombin. Detection of the hybridization was accomplished by monitoring change of the guanine oxidation signal of TBA modified GO/PGE before and after interaction with thrombin. In our strategy, the biosensor presented a detection range from 0.1 nM to 10 nM with a linear coefficient of 0.998, and the detection limit was 0.07 nM. The proposed biosensor could be utilized for the selective and sensitive detection of thrombin in biological samples.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2016.07.053.

References

Bang, G.S., Cho, S., Lee, N., Lee, B.R., Kim, J.H., Kim, B.G., 2013. Biosens. Bioelectron. 39, 44–50.
Bichler, J., Heit, J.A., Owen, W.G., 1996. Thromb. Res. 84, 289–294.
Chen, Y.L., Nakamoto, K.H., Niwa, O., Corn, R.M., 2012. Langmuir 28, 8281–8285.
Chen, Z.B., Li, L.D., Mu, X.J., Zhao, H.T., Guo, L., 2011. Talanta 85, 730–735.
Cote, L.J., Kim, F., Huang, J., 2009. J. Am. Chem. Soc. 131, 1043–1049.
Ellington, A.D., Szostak, J.W., 1990. Nature 346, 818–822.
Francois, L., David, F.W., 1954. Phys. Rev. 34, 722–729.
Geim, A.K., Novoselov, K.S., 2007. Nat. Mater. 6, 183–191.
Guo, S., Du, D.X., Tang, L.N., Ning, Y., Yao, Q.F., Zhang, G.J., 2013. Analyst 138, 3120–3216.
He, Y., Wang, Z.G., Tang, H.W., Pang, D.W., 2011. Biosens. Bioelectron. 29, 76–81.
Holland, C.A., Henry, A.T., Whinna, H.C., Church, F.C., 2000. FEBS Lett. 484, 87–91.
Huang, C.-C., Huang, Y.-F., Cao, Z., Tan, W., Chang, H.-T., 2005. Anal. Chem. 77, 5735–5741.
Huang, H., Zhu, J.J., 2009. Biosens. Bioelectron. 25, 927–930.
Ikebukuro, K., Kiyohara, C., Sode, K., 2005. Biosens. Bioelectron. 20, 2168–2172.
Kang, Y., Feng, K.J., Chen, J.W., Jiang, J.H., Shen, G.L., Yu, R.Q., 2008. Bioelectrochemistry 73, 76–81.
Lee, S.J., Youn, B.-S., Park, J.W., Niazi, J.H., Kim, Y.S., Gu, M.B., 2008. Anal. Chem. 80, 2867–2873.
Li, X.X., Shen, L.H., Zhang, D.D., Qi, H.L., Gao, Q., Ma, F., Zhang, C.X., 2008. Biosens. Bioelectron. 23, 1624–1630.
Liss, M., Petersen, B., Wolf, H., Prohaska, E., 2002. Anal. Chem. 74, 4488–4495.
Nishino, A., Suzuki, M., Ohtani, H., Motohashi, O., Umezawa, K., Nagura, H., Yoshimoto, T., 1993. J. Neurotrauma 10, 167–179.
Pavlov, V., Shlyahovsky, B., Willner, I., 2005. J. Am. Chem. Soc. 127, 6522–6523.
Qin, H.X., Liu, J.Y., Chen, C.G., Wang, J.H., Wang, Ek, 2012. Anal. Chim. Acta 712, 127–131.
Radi, A., Sanchez, J.L.A., Baldrich, E., O'Sullivan, C.K., 2006. J. Am. Chem. Soc. 128, 117–124.
Rosi, N.L., Mirkin, C.A., 2005. Chem. Rev. 105, 1547–1562.
Sharon, E., Liu, X.Q., Freeman, R., Yehezkeli, O., Willner, I., 2013. Electroanalysis 25,

851–856.
Shuman, M.A., Majerus, P.W., 1976. J. Clin. Investig. 58, 1249–1258.
Song, Y., Yang, X., Li, Z., Zhao, Y., Fan, A., 2014. Biosens. Bioelectron. 51, 232–237.
Song, Z.L., Zhao, X.H., Liu, W.N., Ding, D., Bian, X., Liang, H., Zhang, X.B., Chen, Z., Tan, W.H., 2013. Small 9, 951–957.
Wang, J., Jiang, Y., Zhou, C., Fang, X., 2005. Anal. Chem. 77, 3542–3546.
Wang, J., Munir, A., Li, Z., Zhou, H.S., 2009. Biosens. Bioelectron. 25, 124–129.
Wang, Y.H., Bao, L., Liu, Z.H., Pang, D.W., 2011. Anal. Chem. 83, 8130–8137.
William, S., Hummers, J., Offeman, R., 1958. J. Am. Chem. Soc. 80, 1339–1339.
Wu, J., Fu, Z., Yan, F., Ju, H., 2007. Trends Anal. Chem. 26, 679–688.
Wu, L., Zhang, X.H., Liu, W., Xiong, E.H., Chen, J.H., 2013. Anal. Chem. 85, 8397–8402.
Xu, D., Xu, D., Yu, X., Liu, Z., He, W., Ma, Z., 2005. Anal. Chem 77, 5107–5113.
Yan, F.F., Wang, F., Chen, Z.L., 2011. Sens. Actuators B 160, 1380–1385.
Yan, X., Cao, Z., Kai, M., Lu, J., 2009. Talanta 79, 383–387.
Yuan, Y.L., Liu, G.P., Yuan, R., Chai, Y.Q., Gan, X.X., Bai, L.J., 2013. Biosens. Bioelectron. 42, 474–480.
Zhang, Y.L., Huang, Y., Jiang, J.H., Shen, G.L., Yu, R.Q., 2007. J. Am. Chem. Soc. 129, 15448–15449.
Zhang, Z.X., Wang, Z.J., Wang, X.L., Yang, X.R., 2010. Sens. Actuators B: Chem. 147, 428–433.
Zhao, J., He, X.L., Bo, B., Liu, X.J., Yin, Y.M., Li, G.X., 2012. Biosens. Bioelectron. 34, 249–252.
Zhao, J., Xin, M., Cao, Y., Yin, Y., Shu, Y., Ma, W., 2015. Anal. Chim. Acta 860, 23–28.
Zhu, W.P., Zhao, Z.W., Li, Z., Jiang, J.H., Shen, G.L., Yu, R.Q., 2012. Analyst 137, 5506–5509.
Zhuo, B., Li, Y., Huang, X., Lin, Y., Chen, Y., Gao, W., 2015. Sens. Actuators B 208, 518–524.

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