Biomacromol. J. Vol. 2, No. 1, 28-33, June 2016.

Biomacromolecular Journal
www.bmmj.org

Designing a Label Free Aptasensor for Detection of Methamphetamine

R. Kohzadi[a], A. Molaeirad[a,*], M. Alijanianzadeh[a], N. Kamali[a] and M. Mohtashamifar[b]

[a]*Department of Bioscience and Biotechnology, Malek-Ashtar University of Technology, Tehran, Iran*

[b]*Electronic Research Center, Tehran, Iran*

(Received 10 August 2015, Accepted 27 January 2016)

ABSTRACT

A label-free electrochemical nucleic acid aptasensor for the detection of methamphetamine (MA) by the immobilization of thiolated self-assembled DNA sequences on a gold nanoparticles-chitosan modified electrode is constructed. When MA was complexed specifically to the aptamer, the configuration of the nucleic acid aptamer switched to a locked structure and the interface of the biosensor changed, resulting in a variation of the corresponding peak current of an electrochemical probe ($[Fe(CN)_6]^{3-/4-}$). Two different methods of cyclic voltammetry (CV) and microcantilever were employed to determine MA detection limit. Under the optimized experimental conditions, the presented sensor exhibits a nice specificity towards MA. The detection limits for MA in electrode and microcantilever were obtained 10 and 0.7 nM. The proposed aptasensor can be easily regenerated by the denaturalization of aptamer-target complexes in a heated water bath at 80-90 °C. Besides, this biosensor has a high reproducibility and selectivity, which can be a promising method to detect MA in real samples.

Keywords: Aptasensor, Methamphetamine, Detection limit

INTRODUCTION

The drug abuse and smuggling of methamphetamine (MA) and cocaine have been increased year by year in the world, which has dangerous effects on human health and social security. So detection of MA especially in low concentrations is so important and have been attracted the scientist's attentions. Traditional detection methods are time consuming and expensive [1]. Up to now different MA biosensors have been developed [2-18] that none of them are aptamer based. The best detection limit that has been reported is 10 nM [9]. So it is needed to design and construct a more powerful and fast biosensor with lower detection limit.

An aptamer is a synthetic oligonucleotide that can specifically bind to target molecules [19]. Aptamers can be chemically synthesized with low cost and can be modified chemically easily [20]. Many specific aptamers have been designed for detection of different compounds [2,4,21-68]. The cocaine aptamer has been designed before [69]. In this study this aptamer was used for detection of MA and constructing MA aptasensor.

*Corresponding author. E-mail: molaeirad@gmail.com

EXPERIMENTAL

Electrochemical measurements were based on changes in electrical signal after conformational switching of aptamer induced by analyte [71]. Before measurements, aptasensor was incubated with specified concentrations of targets in room temperature and then was rinsed with PBS and the changes were studied by appropriate apparatus.

For assay of MA in microcantilever, stock MA solution (100 nM) was added to buffer solution that microcontilever was inside it (final MA concentration was 0.7 nM). The assay method was based on resistance changes.

METHODS AND MATERIALS

A cocaine aptamer [69] with sequence of 5'-HS-$(CH_2)_6$ GACAAGGAAAATCCTTCAATGAAGTGGGTC-3' was purchased from Takapoozist Company (Tehran, Iran). Aptamer binding affinity for the MA target was measured

by electrochemistry and Microcantilever both in PBS buffer. MA hydrochloride, chitosane, $HAuCl_4$ and $Na_3C_6H_5O_7.2H_2O$ were purchased from Sigma.

Apparatus

Electrochemistry was performed using a BIPOTENTIOSTAT μSTAT 200 electro-chemical workstation Dropsens Co., Spain) in a three-electrode electrochemical cell containing a platinum wire auxiliary electrode, a modified glassy carbon electrode as working electrode and a Ag/AgCl electrode as reference electrode against which all potentials were measured.

The microcantilever with size of 200 μm (width) × 500 μm (length) × 10 μm (thickness) that was coated with Au is constructed by school of electrical engineering in Malek-e-Ashtar University of Technology.

Preparation of Solutions

Aptamer solution. Aptamer solution was prepared in PBS buffer (20 mM Tris.HCl, pH 7.4, 140 mM NaCl and 5 mM KCl) then the aptamer solution was heated in 65.3 °C for 5 min and afterward the solution was kept in room temperature for 1 h.

MA hydrochloride solution. The MA hydrochloride solution was prepared with double distilled water as solvent and the concentration range was between 1 nM to 40 μM.

Chitosan solution. The 0.2% chitosan solution was prepared with acetic acid as solvent. Then the solution was stirred in room temperature for 6 h.

Preperation of Au Nanoparticles (AuNP)

Before preparation of AuNP, all glass containers were washed with aqua regia and afterward they were washed with double distilled water and let them to be dried. The colloidal gold solutions were prepared by a well-established Frens method of reducing a $HAuCl_4$ aqueous solution with citrate. In brief, a 100 ml of $HAuCl_4$ aqueous solution (0.01%) was stirred and heated to 100 °C. Then 2 ml of $Na_3C_6H_5O_7.2H_2O$ (1%) was added to a concentrated $HAuCl_4$ aqueous solution kept boiling for 17 min in order to obtain burgundy solutions [70]. After cooling down, the synthesized gold colloids were stored at 4 °C in a dark bottle.

Preperation of Piranha Solution for Washing Cantilever Surface and Removing Pollutions from Au Surface

This solution was prepared with 70% H_2SO_4 and 30% H_2O_2.

Preparation of Modified Microcantilever Buffer

The PBS buffer with concentration of 4 mM (20 mM Tris.HCl, pH 7.4, 140 mM NaCl and 5 mM KCl) was prepared.

Preparation of Microcantilever-based Biosensor

First the Au-modified cantilever surface was rinsed with fresh Piranha solution to remove pollutions. Then the surface was washed with distilled water and let it to be dried. Second the aptamer solution with concentration of 0.5 μM was added to microcantilever surface and incubated for 3 h that aptamers will be bound to Au-modified surface. Third the 6-mercaptohexanol solution (3 mM) was added to modified miocrocantilever surface and incubated for 1 h to block any non-specificity site and also to separate any loosely bounded aptamer. After these steps the micro-cantilever based biosensor is ready to use and was kept it in the buffer.

Assay Solution

The assay solution was a 10 mM phosphate buffer solution (PBS, pH 7.4) with 5 mM $[Fe(CN)_6]^{3-/4-}$, which was prepared with 10 mM KH_2PO_4, 10 mM Na_2HPO_4, 5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN)_6$, 0.1 M KCl and 0.1 M NaCl.

The Preparation of Aptamer/AuNPs/Chitosan/GCE

A glassy carbon electrode (GCE) was first polished with alumina slurry, successively rinsed thoroughly with absolute alcohol and distilled water in an ultrasonic bath, and dried in air. Then, 10 μl of a CHIT solution was pipetted to the surface of GCE, and remained over night. A CHIT-modified electrode was immersed in a colloidal gold solution for about 12 h in order to adsorb AuNPs. Then, the obtained AuNPs-modified electrode was incubated in a solution of 2 μM aptamer for about 1 h at room temperature. After washing with doubly distilled water, 10 μl mercaptoethanol (MCE) was added on the modified electrode surface to

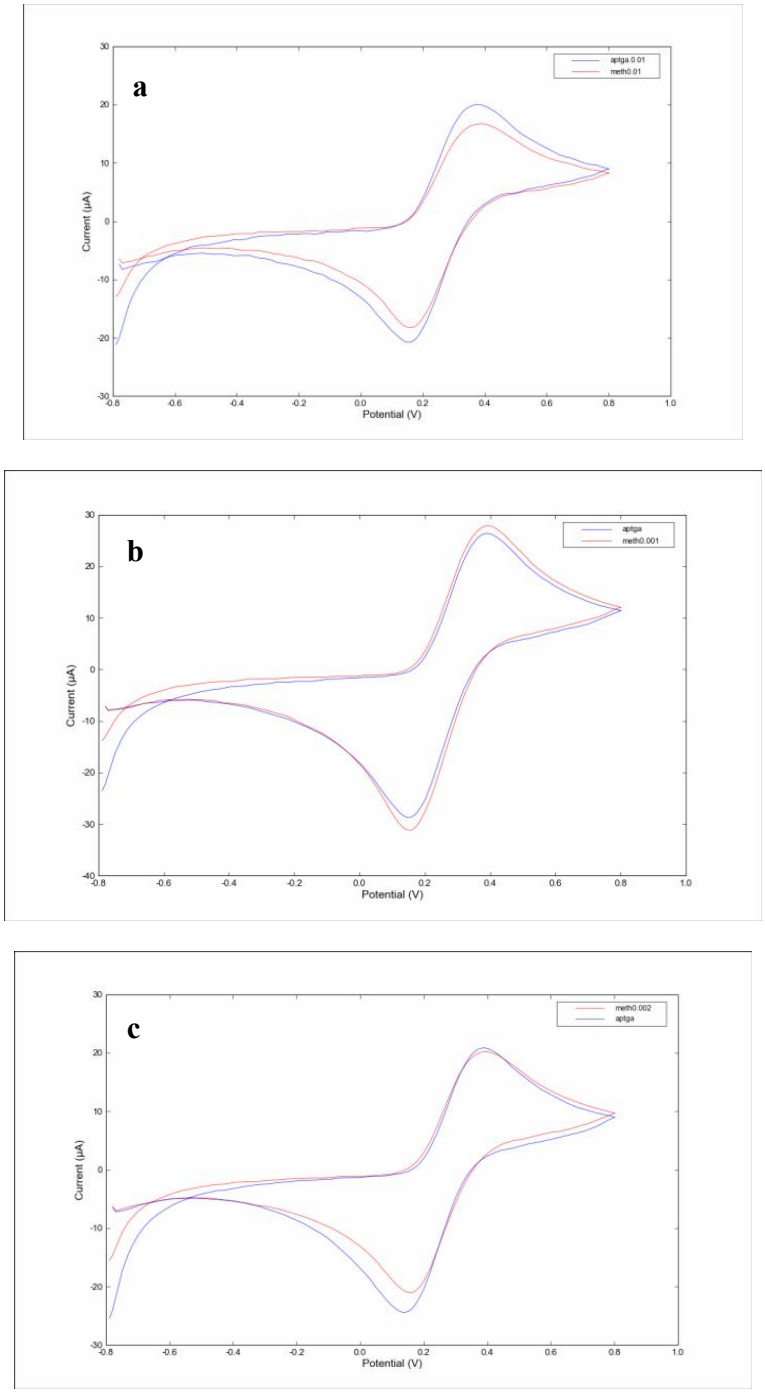

Fig. 1. CVs of different modified electrodes in a 10 mM phosphate buffer solution (PBS pH 7.4) with 5 mM $[Fe(CN)_6]^{3-/4-}$ and 50 mM glucose. a) Blue: aptamer/AuNPs/CHIT/GCE; red: aptamer/AuNPs/CHIT/GCE reacted with 10 mM MA. b) Blue: aptamer/AuNPs/CHIT/GCE; red: aptamer/Au NPs/CHIT/GCE reacted with 1 nM MA. **c)** Blue: aptamer/AuNPs/CHIT/GCE; red: aptamer/AuNPs/CHIT/GCE reacted with 2 nM MA.

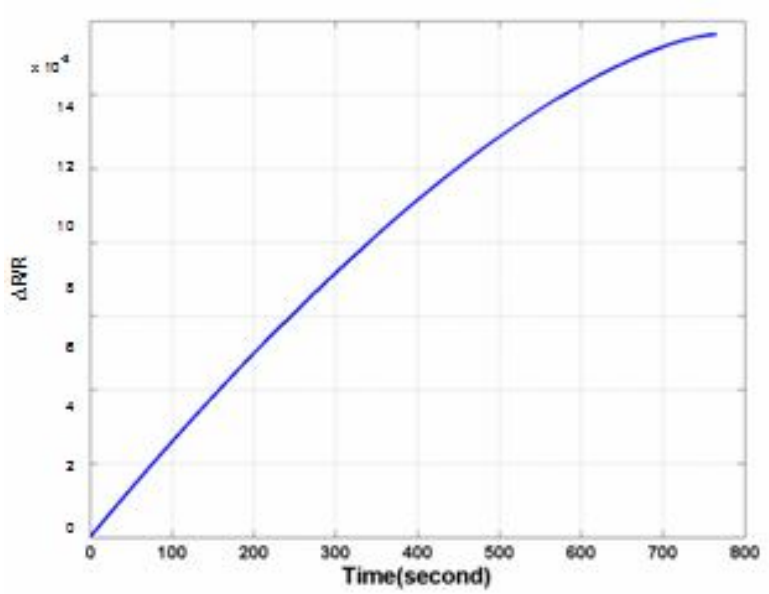

Fig. 2. Plot of resistance changes against time for microcantilever-based biosensor.

block any non-specificity site and also to separate any loosely bounded aptamer. The prepared aptasensor was kept in PBS at 4 °C when not in use.

Regeneration of Aptasensors

For separating the analyte from aptamer, the modified electrode was immersed in hot distilled water (80-90 °C) for 10 min. Then the regenerated transducer interface of aptasensor was rinsed with distilled water and before incubated with target let it to be cooled in room temperature.

RESULTS AND DISCUSSION

Electrochemical Results

Cyclic voltammetric behavior of the aptasensor. The AuNPs-modified electrode and aptamer in the absence and presence of different concentrations of MA (in presence of glucose) (Figs. 1a, b and c) was studied.

As Fig. 1a shows, in the presence of MA (10 nM) the current obviously declined, due to an electrostatic repulsion between negatively charged backbones of the aptamer and the electroactive probe. A reasonable explanation is that the configurations of nucleic acid aptamers shifted when the aptamers reacted with MA, which hindered the diffusion of $[Fe(CN)_6]^{3-/4-}$. As Fig. 1b shows in concentration of 1 nM the current is not decreased. In concentration of 2 nM MA (Fig. 1c), little decrease was occurred that was not significant.

A control experiment of the Au NPs/chitosan/GCE without an aptamer was performed. No obvious current variation was found, which confirms the specific effect of the analyte and the aptamer (data not shown). As figures show, the designed aptasensor can detect MA in the concentration of 10 nM.

In another part the prepared microcontilever was used for detection of MA. The MA solution (100 nM) was added to 5 ml buffer solution that contains microcontilever (final concentration of 0.7 nM) then the results were analyzed. According to Eq. (1) the assay was based on resistance change (Fig. 2).

$$dR/R_0 = (1 + 2\upsilon)/E \times \sigma \quad (1)$$

In this equation, υ is poisson coefficient, σ is tension and dR/R_0 is resistance change to initial resistance. Generally in this study, the change in the mass transfer resistance of a reporter molecule, $[Fe(CN)_6]^{3-/4-}$, to the electrode surface to

monitor the changes in the conformation of DNA aptamers in the presence of MA was used to construct label-free aptamer-based sensors. In designed aptasensor when MA was present, the aptamer self-assembled on gold nanoparticles-modified electrode folds to bind the MA, and as such created a change in the charge structure and sterics at the electrode surface, which in turn changed (increased) the mass-transfer resistance of the electrochemical probe, together with the change in the electrical signal. The decline in the peak current, showed good linearity with the concentration of MA. In another part, aptamer-functionalized microcantilever was used for detection of MA that the results show that resistance change to initial resistance ratio is increased with time.

This proposed electrochemical aptasensor for detecting MA is label-free, controllable, reproducible, effective and easy to prepare, together with high selectivity, which can be extended to being applied in clinic diagnostics and forensic analysis.

REFERENCES

[1] A.G. Verstraete, Ther. Drug Monit. 26 (2004) 200.

[2] X. Ma *et al.*, Biosens. Bioelectron. 55 (2014) 412.

[3] J. Nie, D.-W. Zhang, C. Tie, Y.-L. Zhou, X.-X. Zhang, Biosens. Bioelectron. 49 (2013) 236.

[4] Y. Li, H. Qi, Y. Peng, J. Yang, C. Zhang, Electrochem. Commun. 9 (2007) 2571.

[5] Q. Sheng, R. Liu, S. Zhang, J. Zheng, Biosens. Bioelectron. 51 (2014) 191.

[6] L. Asturias-Arribas, M.A. Alonso-Lomillo, O. Domínguez-Renedo, M.J. Arcos-Martínez, Talanta 105 (2013) 131.

[7] B. Wenger, K. Kugelbrey, H. Gao, H. Sigrist, G. Voirin, Biosens. Bioelectron. 34 (2012) 94.

[8] P.J. Devine *et al.*, Anal. Biochem. 227 (1995) 216.

[9] L.-Y. Zhang, Y.-J. Liu, Anal. Chim. Acta 806 (2014) 204.

[10] A. Choodum *et al.*, Forensic Sci. Int.

[11] G. Man, B. Stoeber, K. Walus, Forensic Sci. Int. 189 (2009) 1.

[12] Y.-C. Tseng, J.-S. Chang, S. Lin, S.D. Chao, C.-H. Liu, Sensor. Actuat. A-Phys. 182 (2012) 163.

[13] G. Sakai, S. Nakata, T. Uda, N. Miura, N. Yamazoe, Electrochim. Acta 44 (1999) 3849.

[14] C. Zhang, G. Feng, Z. Gao, Biosens. Bioelectron. 12 (1997) 1219.

[15] H. Dai, Y. Wang, X. Wu, L. Zhang, G. Chen, Biosens. Bioelectron. 24 (2009) 1230.

[16] N. Miura *et al.*, Sensor. Actuat. B-Chem. 13 (1993) 188.

[17] T. Uda *et al.*, J. Fermentation and Bioengineering 83 (1997) 333.

[18] A. Ahmad, A. Ramakrishnan, M.A. McLean, A.P. Breau, Biosens. Bioelectron. 18 (2003) 399.

[19] D. Shangguan *et al.*, Proceedings of the National Academy of Sciences 103 (2006) 11838.

[20] Q. Yang, I.J. Goldstein, H.-Y. Mei, D.R. Engelke, Proceedings of the National Academy of Sciences 95 (1998) 5462.

[21] Y. Chai, D. Tian, H. Cui, Anal. Chim. Acta 715 (2012) 86.

[22] J. Chen, Z. Fang, J. Liu, L. Zeng, Food Control 25 (2012) 555.

[23] Q. Chen *et al.*, Biosens. Bioelectron. 26 (2010) 575.

[24] L. Du *et al.*, Sensor. Actuat. B-Chem. 187 (2013) 481.

[25] Z. Fang, W. Wu, X. Lu, L. Zeng, Biosens. Bioelectron.

[26] K. Guo *et al.*, Electrochem. Commun. 13 (2011) 707.

[27] O.S. Kwon, S.J. Park, J. Jang, Biomaterials 31 (2010) 4740.

[28] J. Kwun, S. Yun, L. Park, J.H. Lee, Talanta 119 (2014) 262.

[29] J.A. Lee *et al.*, Sensor. Actuat. B-Chem. 129 (2008) 372.

[30] P. Luo, Y. Liu, Y. Xia, H. Xu, G. Xie, Biosensor. Bioelectron. 54 (2014) 217.

[31] X. Ma *et al.*, J. Microbiol. Meth.

[32] J.-W. Park *et al.*, Biosens. Bioelectron. 38 (2012) 233.

[33] R.B. Queirós, N. de-los-Santos-Álvarez, J.P. Noronha, M.G.F. Sales, Sensor. Actuat. B-Chem. 181 (2013) 766.

[34] A. Qureshi, Y. Gurbuz, J.H. Niazi, Procedia Engineering 5 (2010) 828.

[35] C. Shi, H. Gu, C. Ma, Anal. Biochem. 400 (2010) 99.

[36] H. Shu, W. Wen, H. Xiong, X. Zhang, S. Wang,

Electrochem. Commun. 37 (2013) 15.
[37] W. Su *et al.*, Biosens. Bioelectron. 32 (2012) 32.
[38] K. Wang, Z.-H. Tao, L. Xu, Y.-Q. Liu, Chinese J. Anal. Chem. 42 (2014) 298.
[39] S.K. Yadav, B. Agrawal, P. Chandra, R.N. Goyal, Biosens. Bioelectron. 55 (2014) 337.
[40] F. Yan, F. Wang, Z. Chen, Sensor. Actuat. B-Chem. 160 (2011) 1380.
[41] J. Zhang *et al.*, Biosens. Bioelectron. 26 (2011) 2645.
[42] X. Zhu *et al.*, Anal. Chim. Acta 684 (2011) 121.
[43] J. Ashley, S.F.Y. Li, Biosens. Bioelectron. 48 (2013) 126.
[44] L. Barthelmebs, A. Hayat, A.W. Limiadi, J.-L. Marty, T. Noguer, Sensor. Actuat. B-Chem. 156 (2011) 932.
[45] L. Bonel, J.C. Vidal, P. Duato, J.R. Castillo, Biosens. Bioelectron. 26 (2011) 3254.
[46] Z. Chen, L. Chen, H. Ma, T. Zhou, X. Li, Biosens. Bioelectron. 48 (2013) 108.
[47] Z. Chen *et al.*, Biosens. Bioelectron. 56 (2014) 46.
[48] K. Han, L. Chen, Z. Lin, G. Li, Electrochem. Commun. 11 (2009) 157.
[49] R. Hu *et al.*, Biosens. Bioelectron. 53 (2014) 384.
[50] H. Huang, J.-J. Zhu, Biosens. Bioelectron. 25 (2009) 927.
[51] L. Huang *et al.*, Anal. Chim. Acta 787 (2013) 203.
[52] L. Kashefi-Kheyrabadi, M.A. Mehrgardi, Bioelectrochemistry 94 (2013) 47.
[53] Y.H. Kim, J.P. Kim, S.J. Han, S.J. Sim, Sensor. Actuat. B-Chem. 139 (2009) 471.
[54] L. Li, H. Zhao, Z. Chen, X. Mu, L. Guo, Biosens. Bioelectron. 30 (2011) 261.
[55] Y. Li, J. Bao, M. Han, Z. Dai, H. Wang, Biosens. Bioelectron. 26 (2011) 3531.
[56] W. Liao, X.T. Cui, Biosens. Bioelectron. 23 (2007) 218.
[57] Z. Lin *et al.*, Talanta 103 (2013) 371.
[58] A. Oguro, T. Ohtsu, Y. Nakamura, Anal. Biochem. 388 (2009) 102.
[59] A.R. Ruslinda *et al.*, Biosens. Bioelectron. 26 (2010) 1599.
[60] W. Wang, C. Chen, M. Qian, X.S. Zhao, Anal. Biochem. 373 (2008) 213.
[61] W. Wang, C. Chen, M.X. Qian, X.S. Zhao, Sensor. Actuat. B-Chem. 129 (2008) 211.
[62] L. Xie, L. You, X. Cao, Spectrochim. Acta Part A: Mol. Biomol. Spectroscopy 109 (2013) 110.
[63] S. Xie, S.P. Walton, Biosens. Bioelectron. 25 (2010) 2663.
[64] H. Yang, J. Ji, Y. Liu, J. Kong, B. Liu, Electrochem. Commun. 11 (2009) 38.
[65] C. Yao *et al.*, Biosens. Bioelectron. 24 (2009) 2499.
[66] W. Yao, L. Wang, H. Wang, X. Zhang, L. Li, Biosens. Bioelectron. 24 (2009) 3269.
[67] J. Zhang *et al.*, Electrochem. Commun. 25 (2012) 5.
[68] B. Zheng, S. Cheng, W. Liu, M.H.-W. Lam, H. Liang, Anal. Biochem. 438 (2013) 144.
[69] J. Liu, D. Mazumdar, Y. Lu, Angew. Chem. Int. Edit. 45 (2006) 7955.
[70] Z.Q.G.R.Q. Feng, J.B. Mi, Modern Technology and Application of Antibody. Peking University, China, ed. Peking University, 2006.
[71] M.N. Stojanovic, D.W. Landry, J. Am. Chem. Soc. 124 (2002) 9678.