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A novel electrochemical aptasensor based on nontarget-induced high accumulation of methylene blue on the surface of electrode for sensing of α-synuclein oligomer

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ABSTRACT

This study describes a novel electrochemical aptasensor for detection of α-synuclein (α-syn) oligomer, an important biomarker related to Parkinson's and Alzheimer's diseases. The sensing platform is based on exonuclease I (Exo I), terminal deoxynucleotidyl transferase (TdT) and methylene blue. The aptasensor exploits the improved sensitivity because of applications of TdT and Exo I and also a label-free aptamer (Apt). Furthermore, direct immobilization of complementary strand of aptamer (CS) instead of Apt on the surface of electrode prohibits Apt self-assembled monolayer aggregation and keeps the function of the Apt. In the absence of α-syn oligomer, TdT enhances lengths of Apt and CS and so, increases accumulation of methylene blue as redox agent on the surface of electrode, leading to a strong current signal. While in the presence of α-syn oligomer, Exo I digests CS on the electrode surface, resulting in less accumulation of methylene blue on the electrode surface and a weak current signal. The relative electrochemical signal of the aptasensor increased linearly with the logarithm of α-syn oligomer concentration in the range from 60 pM to 150 nM. The detection limit was 10 pM. Furthermore, the sensor showed high precision and repeatability for detection of α-syn oligomer in serum samples.

1. Introduction

Parkinson's disease and dementia are the most prevalent neurodegenerative disorders in the world (Cremades et al., 2017). They are characterized by α-synuclein (α-syn) oligomers which are commonly found in the plasma and cerebrospinal fluid of patients (Ayoobi et al., 2017; Tsukakoshi et al., 2012; Zheng et al., 2018). Thus, sensing of α-syn oligomer has great potential for early diagnosis of these neurodegenerative diseases.

Currently, the reliable determination of α-syn oligomer depends on instrumental detection, such as atomic force microscopy, mass spectrometry and capillary electrophoresis. These analytical techniques are usually expensive and need complex and time-consuming sample pretreatment and skilled operators (Leung et al., 2015; Sun et al., 2017). So, it is vital to develop analytical methods for detection of α-syn oligomer which are simple and more economical.

Recently, aptamers as recognition elements have obtained increasing interest in the development of analytical methods. Aptamers are functional single-stranded oligonucleotides isolated from random-sequence nucleic acid libraries through Systematic Evolution of Ligands by EXponential enrichment (SELEX) in vitro (Geng et al., 2018; Niazi et al., 2018). They can recognize a broad range of target analytes with high affinity and selectivity (Hu et al., 2018; Liu et al., 2018). Compared to antibodies, aptamers have some merits, including simple and rapid synthesis, thermal stability, ease of labeling, resistance to denaturation and low cost (Abnous et al., 2018; Yang et al., 2018; Zejli et al.,

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2018).

Over the last few years, electrochemical aptasensors have received increasing attention due to their superior features, including miniaturization of the electrochemical instruments, high sensitivity and simple operation (Abnous et al., 2017; Ensafi et al., 2018; Jiang et al., 2018).

In this contribution, a novel electrochemical aptasensor was introduced for detection of α-syn oligomer based on screen-printed gold electrode (SPGE), methylene blue, exonuclease I (Exo I) and nontarget-induced increase of size of aptamer (Apt) and its complementary strand (CS) triggered by terminal deoxynucleotidyl transferase (TdT). In this work, CS was covalently immobilized on the surface of SPGE. So that, Apt could use its CS to attach on the surface of electrode, leading to increase of the affinity and function of Apt toward its target due to application of label-free aptamer and prevention of Apt self-assembled monolayer aggregation (Sun et al., 2018a). In addition, application of TdT led to high accumulation of methylene blue as redox agent on the electrode surface in the absence of target, while use of Exo I resulted in very low presence of methylene blue on the surface of electrode in the presence of α-syn oligomer, leading to improvement of the sensitivity of the designed aptasensor. Moreover, SPGE allowed portable and feasible in situ detection of α-syn oligomer.

2. Materials and methods

2.1. Materials

α-syn oligomer (Apt) (Sun et al., 2017; Tsukakoshi et al., 2012), 5′-TTTTTGGTGGCTGGAGGGGGCGCGAACG-3′, and its complementary strand (CS), 5′-Thiol-TTCCCGTTCGCGCCCCCTCC-3′, were synthesized by Bioneer (South Korea, the underlined sequences are complementary regions). Recombinant human α-synuclein (α-syn) protein aggregation and human cardiac troponin I protein were obtained from Abcam (United Kingdom). 2′-deoxythymidine 5′-triphosphate (dTTP), human serum albumin (HSA), methylene blue, human serum, immunoglobulin G (IgG), Tris(2-carboxyethyl) phosphine hydrochloride (TCEP) and 6-mercaptohexanol (MCH) were purchased from Sigma-Aldrich (USA). Platelet-derived growth factor-BB (PDGF-BB) and human myoglobin were purchased from ProSpec (USA). Exonuclease I and terminal deoxynucleotidyl transferase (TdT) were ordered from Thermo Fisher Scientific (USA).

2.2. Apparatus and instrumentation

Atomic force microscopy (AFM) and scanning electron microscopy (SEM) images were recorded on a JPK Nanowizard II microscope (Germany) and TESCAN MIRA3 microscope (Czech Republic), respectively.

The electrochemical detections were performed on disposable screen-printed gold electrodes (SPGEs, DRP-C220BT, Spain) using a μstat 400 portable Biopotentiostat/ Galvanostat (DropSens, Spain). DropView8400 software was used to obtain the values of electrochemical signals.

2.3. Fabrication of the electrochemical aptasensor (Apt-CS-modified electrode)

10 μL TCEP-treated 5′-Thiol CS (1 μM) was immobilized on the surface of electrode for 3 h at room temperature (100% humidity). Then, the unbound CS was thoroughly washed away using ultrapure water. Thereafter, 10 μL Apt (1 μM) was dropped on the surface of electrode and incubated for 90 min at room temperature, followed by rinsing of the electrode surface with ultrapure water. Finally, the blank sites on the surface of electrode were blocked by 1 mM MCH for 1 h.

2.4. Electrochemical measurement of α-syn oligomer

Human serum samples were diluted 10 times for detection of α-syn oligomer. Then, various concentrations of α-syn oligomer (0–300 nM) were added into diluted serum samples. Next, 10 μL of the above mixtures were placed on the surfaces of Apt-CS-modified electrodes for 30 min at room temperature. Thereafter, the electrodes were incubated with 20 U Exo I for 40 min at 37 °C, followed by rinsing of the surfaces of electrodes with ultrapure water. After that, 10 μL reaction mixture containing 15 U TdT, 1X TdT reaction buffer and 10 mM dTTP was placed on the surfaces of electrodes for 75 min at 37 °C. After washing of the surfaces of electrodes, 10 μL methylene blue (120 μM) was dropped on the surfaces of electrodes and incubated for 10 min. Finally, the surfaces of electrodes were washed by ultrapure water and differential pulse voltammetry (DPV) was performed in 10 mM phosphate buffer saline (PBS, pH 7.4) with potential range from − 0.41 V to − 0.31 V, pulse potential of 0.05 V and pulse time of 50 ms.

2.5. Assessment of selectivity of the aptasensor

To determine the selectivity of the sensing platform, the common interfering proteins such as HSA, IgG, myoglobin, PDGF-BB and cardiac troponin I (150 nM the concentration of each substance) were monitored using the designed aptasensor as mentioned above.

3. Results and discussion

3.1. Principle of the aptasensor for α-syn oligomer detection

The principle of the sensing platform for detection of α-syn oligomer has been illustrated in Scheme 1. It is based on target-induced displacement reaction, Exo I as an amplifying element of sensitivity and nontarget-induced extension of Apt and its CS lengths caused by TdT.

Exo I is an enzyme which can selectively hydrolyze single-stranded DNA (ssDNA) in the direction of 3–5′ (Huang et al., 2018; Sun et al., 2018b). TdT is a DNA polymerase which catalyzes the addition of mononucleotides to the 3′-OH end of DNA molecules, leading to formation of long ssDNA (Guo et al., 2018; Luo et al., 2017).

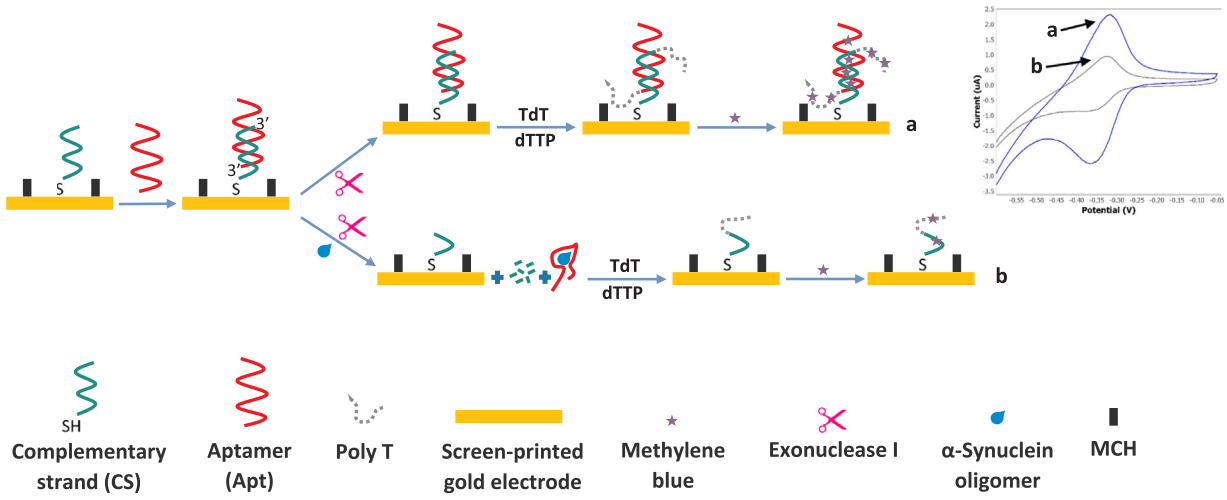
5′-Thiol CS is covalently bound to SPGE using Au-S bond. Then, Apt is immobilized on the surface of electrode through its Watson crick hybridization with CS (Fig. S1, lane 3). In the presence of α-syn oligomer, target-induced displacement reaction occurs and Apt-Target complex leaves the CS (Fig. S1, lane 6) and the surface of electrode. So, Exo I can digest 3′-end of CS on the surface of electrode (Fig. S1, lane 7), leading to low accumulation of methylene blue on the electrode surface following the addition of TdT and a weak electrochemical signal.

In the absence of α-syn oligomer, dsDNA (Apt/CS) remains intact and, so that, is protected against Exo I digestion (Fig. S1, lane 4). Thus, TdT can catalyze poly T generation at the 3′-terminus of CS and Apt, resulting in long ssDNAs on the surface of electrode (Fig. S1, lane 5). Therefore, methylene blue as a positively charged redox agent can interact with these long ssDNAs and also dsDNA on the SPGE surface through both electrostatic force and intercalating binding, resulting in a strong current response.

3.2. Optimization of the experimental factors

The electrochemical performance of the sensing platform can be affected by different parameters, including the concentration of Exo I and incubation time of TdT. To obtain the best analytical performance of the sensing platform, these parameters were optimized.

To examine the influence of Exo I concentration on the response of aptasensor, various concentrations of Exo I (5–30 U) were dropped on the surfaces of Apt-CS-modified electrodes treated with α-syn oligomer. Then, the relative electrochemical signals were recorded using DPV. Fig. 1(a) shows that relative electrochemical signal increased with the



Scheme 1. Schematic illustration of the assembly of the Apt-CS modified electrode and the principle of the electrochemical aptasensor for detecting α-synuclein oligomer.

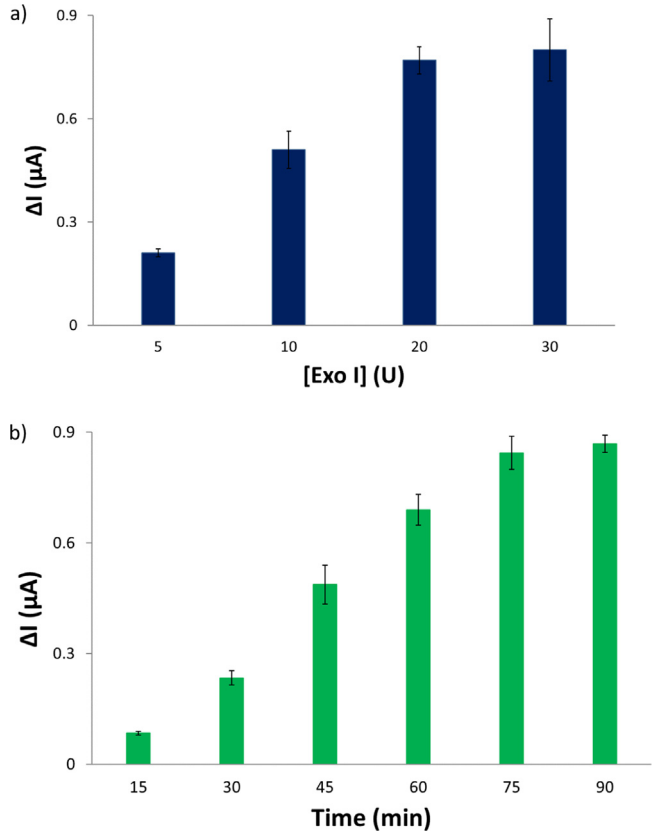


Fig. 1. Optimization of the experimental parameters. (a) Effect of Exo I concentration on the relative electrochemical response (I_0 - I) of Apt-CS-modified electrodes treated with α-syn oligomer. I_0 and I are the current signals before and after addition of Exo I. (b) Relative electrochemical signals (I - I_0) of Apt-CS-modified electrodes treated with Exo I at various incubation time of TdT. I_0 and I are the current signals before and after addition of TdT.

increase of concentration of Exo I and reached plateau at 20 U Exo I. Therefore, this concentration was chosen for the next experiments.

Incubation time of TdT was another important factor which was optimized. Apt-CS-modified electrodes treated with Exo I were incubated with 15 U TdT with varying incubation times from 15 to 90 min. The relative electrochemical signal reached plateau at 75 min (Fig. 1(b)). Thus, 75 min incubation time of TdT was adopted for other experiments.

3.3. Electrochemical characterization of Apt-CS-modified electrode and evaluation of the feasibility of the aptasensor

The cyclic voltammetry (CV) was employed to characterize the various fabrication steps of the aptasensor (Fig. 2). After the modification of SPGE with CS, the peak current was observed (orange curve, e curve) because the positively charged methylene blue as redox agent interacted with negatively charged CS by electrostatic forces, confirming covalent binding of CS on the surface of electrode. Addition of Apt further enhanced the redox peak (green curve, b curve), owing to the more loading of methylene blue on the surface of electrode via its interaction with dsDNA (Apt/CS) on the surface of electrode, suggesting the successful capture of Apt and its hybridization with CS on the

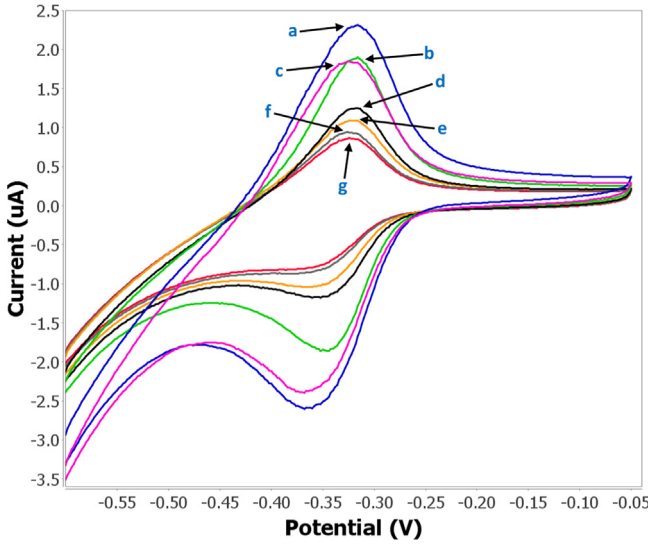


Fig. 2. Electrochemical characterization of the sensing platform fabrication and function. CS-modified electrode (orange curve, e curve), Apt-CS-modified electrode (green curve, b curve), Apt-CS-modified electrode + Exo I (pink curve, c curve), Apt-CS-modified electrode + Exo I + TdT (blue curve, a curve), Apt-CS-modified electrode + α-syn oligomer (black curve, d curve), Apt-CS-modified electrode + α-syn oligomer + Exo I (red curve, g curve), Apt-CS-modified electrode + α-syn oligomer + Exo I + TdT (gray curve, f curve). CV measurements were carried out in PBS solution, scanning from − 0.6 V to − 0.05 V at a scan rate of 100 mV/s.

electrode surface. In the absence of α-syn oligomer, the signal response slightly changed after the Apt-CS-modified electrode was incubated with Exo I (pink curve, c curve), showing the protection of dsDNA structure on the surface of electrode against Exo I digestion. However, in the presence of TdT, the electrochemical signal increased greatly (blue curve, a curve) because of the elongation of the lengths of both Apt and CS on the surface of electrode and accumulation of more methylene blue on the electrode surface. In the presence of α-syn oligomer, the redox current significantly decreased (black curve, d curve) compared to current signal of Apt-CS(dsDNA)-modified electrode (green curve, b curve) due to the formation of Apt-Target complex, leading to release of Apt from CS and electrode surface and presence of less amounts of methylene blue on the electrode surface. In this situation, upon addition of Exo I, the current signal was further reduced (red curve, g curve) owing to the digestion of CS as a ssDNA on the surface of electrode via Exo I which triggered less accumulation of methylene blue on the surface of electrode. Addition of TdT slightly changed the peak current because low amounts of oligonucleotides existed on the electrode surface (gray curve, f curve).

The results of gel retardation assay (Fig. S1) which were mentioned in the sensing scheme, also confirmed the function of the presented aptasensor.

Moreover, the immobilization of CS on the surface of electrode was further analyzed through investigation of morphology and roughness of electrode surface by SEM and AFM, respectively. The results showed that the morphology of bare electrode changed following the addition of CS (Fig. S2). Also, the roughness of electrode increased following the treatment with CS (Fig. S3). All these results verified the immobilization of CS on the electrode surface (Srivastava et al., 2018; Yang et al., 2018).

3.4. Quantitation of α-syn oligomer

Under optimized conditions, the DPV measurements were performed. The presented aptasensor was incubated with serum samples containing various concentrations of α-syn oligomer to quantitatively analyze the response of the sensing platform toward α-syn oligomer. As indicated in Fig. 3(a), the DPV peak current decreased with the increase of α-syn oligomer concentrations. The sensing platform displayed a wide linear relationship between the relative electrochemical signal and the logarithm of α-syn oligomer concentrations in the range of 60 pM to 150 nM (Fig. 3(b)), with limit of detection (LOD) of 10 pM (S/N = 3). Relative to a colorimetric aptasensor using gold nanoparticles for α-syn oligomer detection (LOD of 10 nM) (Sun et al., 2017), the designed sensing platform was superior in sensitivity.

3.5. Selectivity performance of the aptasensor

To determine the aptasensor specificity, the response of aptasensor was evaluated in the presence of different interfering proteins. As shown in Fig. 4, α-syn oligomer (target) caused at least 8-time increase in the relative electrochemical signal compared to other interfering proteins. This proved that the developed sensing platform had good specificity toward α-syn oligomer.

3.6. Recovery assay

To investigate the repeatability and accuracy of the proposed sensing platform, the electrochemical aptasensor was used to determine known concentrations of α-syn oligomer spiked in serum samples. It was found that recoveries changed within the range of 95.3–107% with the relative standard deviations (RSDs) between 1.8% and 4.9% (Table 1). Generally, the acceptable recovery range for aptasensors is between 88% and 110% (Deng et al., 2018; Li et al., 2018; Pan et al., 2018; Zhang et al., 2018). These results offered the designed electrochemical aptasensor has potential to detect α-syn oligomer in real

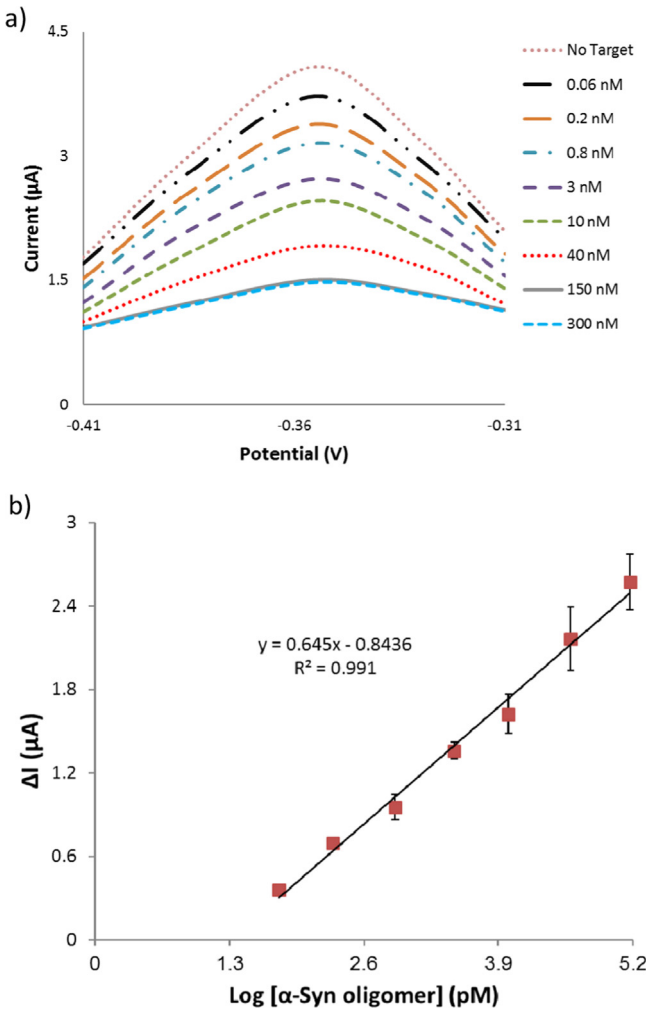


Fig. 3. (a) DPV of the proposed aptasensor in the presence of serum samples containing α-syn oligomer with different concentrations (0–300 nM). (b) The linear relationship of the relative electrochemical signal (I_0 – I) vs. the logarithm of α-syn oligomer concentration. I_0 and I are the current signals before and after addition of α-syn oligomer.

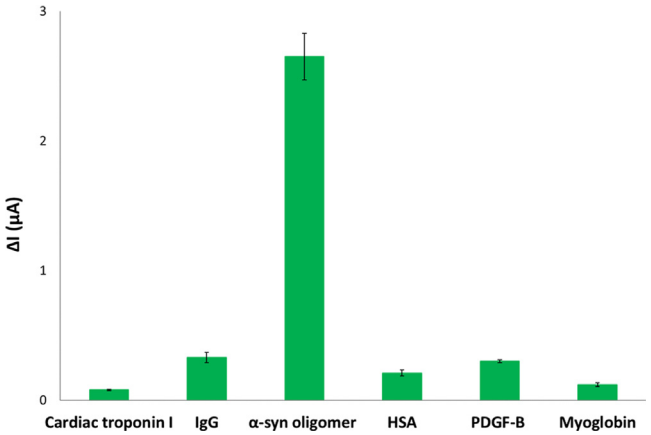


Fig. 4. Selectivity test of the sensing system for α-syn oligomer. The relative electrochemical signal change (I_0 – I) of the sensing system with interfering proteins (the concentration of each substance was 150 nM). I_0 and I are the current signals before and after addition of each substance.

Table 1
Recovery of α-Syn oligomer from human serum samples. Data are mean ± RSD (n = 5).

Serum samples	Added α-Syn oligomer (nM)	Found (nM)	Recovery (%)	RSD (%, n = 5)
1	2	2.1	105	4.9
2	10	10.7	107	4.9
3	30	28.6	95.3	3.6
4	100	98.6	98.6	1.8

samples.

3.7. Stability of the sensing platform

The long-term stability of the aptasensor indicated a loss of about 3.5% in response (n = 4) when the designed analytical method was utilized for detection of 20 nM α-Syn oligomer by the modified electrodes which have been stored at 4 °C for 15 days (Fig. S4). These results verified high stability of the proposed analytical method.

4. Conclusion

In summary, a novel electrochemical aptasensor was designed for sensitive detection of α-syn oligomer based on TdT, Exo I, methylene blue and target-induced displacement of Apt. The sensor was designed in a way in which the presence or absence of α-syn oligomer led to accumulation of very different amounts of methylene blue as redox agent on the surface of electrode. Also, applications of TdT and Exo I could significantly improve the sensitivity of the developed sensing method. The aptasensor showed high selectivity toward α-syn oligomer with a detection limit of 10 pM. Moreover, the presented electrochemical aptasensor was successfully applied for precise and repeatable analysis of serum samples containing different concentrations of α-syn oligomer, showing the fabricated aptasensor can provide a new sensing platform for detection of α-syn oligomer in real samples. However, the use of TdT enzyme enhances the cost and detection time of the developed sensing approach. The developed analytical technique can be easily extended for detection of other proteins by replacements of Apt and its CS.

Acknowledgments

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Conflict of interest

There is no conflict of interest about this article.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2018.09.081

References

Abnous, K., Danesh, N.M., Alibolandi, M., Ramezani, M., Sarreshtehdar Emrani, A., Zolfaghari, R., Taghdisi, S.M., 2017. A new amplified π-shape electrochemical aptasensor for ultrasensitive detection of aflatoxin B_1. Biosens. Bioelectron. 94, 374–379.
Abnous, K., Danesh, N.M., Ramezani, M., Taghdisi, S.M., Emrani, A.S., 2018. A novel colorimetric aptasensor for ultrasensitive detection of cocaine based on the formation of three-way junction pockets on the surfaces of gold nanoparticles. Anal. Chim. Acta 1020, 110–115.
Ayoobi, F., Shamsizadeh, A., Fatemi, I., Vakilian, A., Allahtavakoli, M., Hassanshahi, G., Moghadam-Ahmadi, A., 2017. Bio-effectiveness of the main flavonoids of Achillea millefolium in the pathophysiology of neurodegenerative disorders- a review. Iran. J. Basic Med. Sci. 20 (6), 604–612.
Cremades, N., Chen, S.W., Dobson, C.M., 2017. Structural characteristics of α-synuclein oligomers. Int. Rev. Cell Mol. Biol. 79–143.
Deng, J., Liu, Y., Lin, X., Lyu, Y., Qian, P., Wang, S., 2018. A ratiometric fluorescent biosensor based on cascaded amplification strategy for ultrasensitive detection of kanamycin. Sens. Actuators B: Chem. 273, 1495–1500.
Ensafi, A.A., Amini, M., Rezaei, B., 2018. Molecularly imprinted electrochemical aptasensor for the attomolar detection of bisphenol A. Microchim. Acta 185 (5).
Geng, Y., Guo, M., Tan, J., Huang, S., Tang, Y., Tan, L., Liang, Y., 2018. A fluorescent molecularly imprinted polymer using aptamer as a functional monomer for sensing of kanamycin. Sens. Actuators B: Chem. 268, 47–54.
Guo, J., Yuan, C., Yan, Q., Duan, Q., Li, X., Yi, G., 2018. An electrochemical biosensor for microRNA-196a detection based on cyclic enzymatic signal amplification and template-free DNA extension reaction with the adsorption of methylene blue. Biosens. Bioelectron. 105, 103–108.
Hu, X., Goud, K.Y., Kumar, V.S., Catanante, G., Li, Z., Zhu, Z., Marty, J.L., 2018. Disposable electrochemical aptasensor based on carbon nanotubes- V2O5-chitosan nanocomposite for detection of ciprofloxacin. Sens. Actuators B: Chem. 268, 278–286.
Huang, S., Gan, N., Li, T., Zhou, Y., Cao, Y., Dong, Y., 2018. Electrochemical aptasensor for multi-antibiotics detection based on endonuclease and exonuclease assisted dual recycling amplification strategy. Talanta 179, 28–36.
Jiang, D., Du, X., Zhou, L., Li, H., Wang, K., 2018. TiO2 nanoparticles embedded in borocarbonitrides nanosheets for sensitive and selective photoelectrochemical aptasensing of bisphenol A. J. Electroanal. Chem. 818, 191–197.
Leung, C.W.T., Guo, F., Hong, Y., Zhao, E., Kwok, R.T.K., Leung, N.L.C., Chen, S., Vaikath, N.N., El-Agnaf, O.M., Tang, Y., Gai, W.P., Tang, B.Z., 2015. Detection of oligomers and fibrils of α-synuclein by AIEgen with strong fluorescence. Chem. Commun. 51 (10), 1866–1869.
Li, L., Li, Q., Liao, Z., Sun, Y., Cheng, Q., Song, Y., Song, E., Tan, W., 2018. Magnetism-resolved separation and fluorescence quantification for near-simultaneous detection of multiple pathogens. Anal. Chem. 90 (15), 9621–9628.
Liu, R., Wu, H., Lv, L., Kang, X., Cui, C., Feng, J., Guo, Z., 2018. Fluorometric aptamer based assay for ochratoxin A based on the use of exonuclease III. Microchim. Acta 185 (5).
Luo, L., Xu, F., Shi, H., He, X., Qing, T., Lei, Y., Tang, J., He, D., Wang, K., 2017. Label-free and sensitive assay for deoxyribonuclease I activity based on enzymatically-polymerized superlong poly(thymine)-hosted fluorescent copper nanoparticles. Talanta 169, 57–63.
Niazi, S., Wang, X., Pasha, I., Khan, I.M., Zhao, S., Shoaib, M., Wu, S., Wang, Z., 2018. A novel bioassay based on aptamer-functionalized magnetic nanoparticle for the detection of zearalenone using time resolved-fluorescence NaYF4:Ce/Tb nanoparticles as signal probe. Talanta 186, 97–103.
Pan, J., Li, Q., Zhou, D., Chen, J., 2018. Ultrasensitive aptamer biosensor for arsenic (III) detection based on label-free triple-helix molecular switch and fluorescence sensing platform. Talanta 189, 370–376.
Srivastava, M., Nirala, N.R., Srivastava, S.K., Prakash, R., 2018. A comparative study of aptasensor vs immunosensor for label-free PSA cancer detection on GQDs-AuNRs modified screen-printed electrodes. Sci. Rep. 8 (1), 1923.
Sun, D., Lu, J., Chen, D., Jiang, Y., Wang, Z., Qin, W., Yu, Y., Chen, Z., Zhang, Y., 2018a. Label-free electrochemical detection of HepG2 tumor cells with a self-assembled DNA nanostructure-based aptasensor. Sens. Actuators B: Chem. 268, 359–367.
Sun, K., Xia, N., Zhao, L., Liu, K., Hou, W., Liu, L., 2017. Aptasensors for the selective detection of alpha-synuclein oligomer by colorimetry, surface plasmon resonance and electrochemical impedance spectroscopy. Sens. Actuators B: Chem. 245, 87–94.
Sun, X., Wang, H., Jian, Y., Lan, F., Zhang, L., Liu, H., Ge, S., Yu, J., 2018b. Ultrasensitive microfluidic paper-based electrochemical/visual biosensor based on spherical-like cerium dioxide catalyst for miR-21 detection. Biosens. Bioelectron. 105, 218–225.
Tsukakoshi, K., Abe, K., Sode, K., Ikebukuro, K., 2012. Selection of DNA aptamers that recognize α-synuclein oligomers using a competitive screening method. Anal. Chem. 84 (13), 5542–5547.
Yang, S., Zhang, F., Wang, Z., Liang, Q., 2018. A graphene oxide-based label-free electrochemical aptasensor for the detection of alpha-fetoprotein. Biosens. Bioelectron. 112, 186–192.
Zejli, H., Goud, K.Y., Marty, J.L., 2018. Label free aptasensor for ochratoxin A detection using polythiophene-3-carboxylic acid. Talanta 185, 513–519.
Zhang, G., Li, T., Zhang, J., Chen, A., 2018. A simple FRET-based turn-on fluorescent aptasensor for 17β-estradiol determination in environmental water, urine and milk samples. Sens. Actuators B: Chem. 273, 1648–1653.
Zheng, Y., Qu, J., Xue, F., Zheng, Y., Yang, B., Chang, Y., Yang, H., Zhang, J., 2018. Novel DNA aptamers for parkinson's disease treatment inhibit α-synuclein aggregation and facilitate its degradation. Mol. Ther. - Nucleic Acids 11, 228–242.