Biosensors and Bioelectronics 78 (2016) 154–159

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A label-free kissing complexes-induced fluorescence aptasensor using DNA-templated silver nanoclusters as a signal transducer

Kai Zhang*, Ke Wang, Xue Zhu, Minhao Xie

Key Laboratory of Nuclear Medicine, Ministry of Health, Jiangsu Key Laboratory of Molecular Nuclear Medicine, Jiangsu Institute of Nuclear Medicine, Wuxi, Jiangsu 214063, China

ARTICLE INFO

Article history:
Received 17 October 2015
Received in revised form
11 November 2015
Accepted 13 November 2015
Available online 14 November 2015

Keywords:
Riboswitches
Kissing complexes-induced aptasensor
Adenosine
Adenosine deaminase

ABSTRACT

Riboswitches are complex folded RNA domains that serve as receptors for specific metabolites which identified in prokaryotes. They are comprised of a biosensor that includes the binding site for a small ligand and they respond to association with this ligand by undergoing a conformational change. In the present study, we report on the integration of silver nanoclusters (AgNCs) and riboswitches for the development of a kissing complexes-induced aptasensor (KCIA). We specifically apply the tunable riboswitches properties of this strategy to demonstrate the multiplexes analysis of adenosine and adenosine deaminase (ADA). This strategy allows for simple tethering of the specific oligonucleotides stabilizing the AgNCs to the nucleic acid probes. This is a new concept for aptasensors, and opens an opportunity for design of more novel biosensors based on the kissing complexes-induced strategy.

1. Introduction

Few-atom noble-nanoclustes, such as gold nanoclusters (AuNCs) and silver nanoclusters (AgNCs), are a new class of fluorophores and have great potential for applications in biomedicine (Liu et al., 2012). Especially, AgNCs own an appealing set of features that complements the properties of organic dyes and quantum dots (Zhang et al., 2014a, 2014b). They have desirable photophysical properties and low toxicity suitable for biological applications and have attracted special attention due to their facile synthesis, tunable fluorescence emission, and high photostability (Huang et al., 2011, 2012). In recent years, these new fluorescent materials have been successfully applied to detect various biologically important analyses based on different signal-transducing mechanisms (Huang et al., 2012; Liu et al., 2012; Sharma et al., 2012; Yang and Vosch, 2011; Yeh et al., 2012). Zhu et al. reported a binding-induced fluorescence method using aptamer-functionalized AgNCs probes for the detection of protein (Li et al., 2012). However, this method requires the binding of two affinity probes to the target protein, which limits the universal application.

Retroviruses package two copies of their RNA genome held together near their 5′ end; this is the basis for an efficient recombination process during reverse transcription. This replication process gives the virus an increased capacity to overcome cuts in its RNA genome by allowing the reverse transcriptase to jump from one strand to the other one (Ennifar et al., 2001). The first event leading to dimerization is the formation at the dimerization initiation site (DIS) of a loop–loop complex, also called "kissing complex" (Ennifar et al., 2001; DUCONGÉ et al., 1999), by Watson-Crick pairing of a self-complementary sequence within an apical hairpin loop. In addition, Toulmé et al. selected candidates against RNA hairpins led to stem-loop aptamers in which the loop is complementary to that of the target hairpin, thus generating loop–loop interactions leading to the formation of kissing complexes (Durand et al., 2014). In addition, riboswitches are complex folded RNA domains that serve as receptors for specific metabolites which identified in prokaryotes (Mandal and Breaker, 2004). They are comprised of a biosensor that includes the binding site for a small ligand and they respond to association with this ligand by undergoing a conformational change (Brunel et al., 2002; Mandal and Breaker, 2004). Toulmé et al. reported a riboswitches based on kissing complexes for the detection of small ligands (Durand et al., 2014). They exploited the formation of kissing complexes for sensing the presence of an adenosine or guanosine triphosphate (GTP) that is specifically recognized by a hairpin aptamer. The aptamer is engineered in such a way that the binding of the small molecule shifts its conformation from an unfolded to a folded shape, hence the name aptaswitch. The folded structure is then recognized by a second hairpin, named aptakiss, which is able to form a kissing complex with the aptaswitch. Nevertheless, this strategy requires an immobilized or a fluorescently labeled aptakiss, which will inevitably result in some complicated or expensive operations. Therefore, it is highly desirable to design label-free riboswitches sensors.

* Corresponding author.
E-mail address: zhangkai@jsinm.org (K. Zhang).

http://dx.doi.org/10.1016/j.bios.2015.11.038
0956-5663/

In the present study, we report on the integration of DNA-AgNCs and riboswitches for the development of a kissing complexes-induced aptasensor (KCIA). We specifically apply the tunable riboswitches properties of this strategy to demonstrate the multiplexes analysis of adenosine and adenosine deaminase (ADA). In contrast to related riboswitches-based aptasensors, the present study allows for simple tethering of the specific oligonucleotides stabilizing the AgNCs to the nucleic acid probes.

2. Experimental section

2.1. Materials and chemicals

ADA was purchased from Diazyme Laboratories (La Jolla, CA, USA). Adenosine, guanosine, uridine, and cytidine were obtained from Sigma-Aldrich (St. Louis, MO). Nuclease inhibitor RNasin, and diethypyrocarbonate (DEPC) were ordered from Promega. Oligonucleotide was purchased from Genscript Biotechnology Co., Ltd (Nanjing, China) and listed in Fig. 1. Silver nitrate ($AgNO_3$) were purchased from Sinopharm Chemical Reagent Company (Shanghai, China). Other chemicals were all of analytical grade. All solutions were prepared with Milli-Q (Branstead) purified double distilled water having specific resistance of $>18\ M\Omega$ cm. All oligonucleotide samples were prepared with phosphate buffer (20 mM phosphate, 10 mM magnesium acetate, 1 unit μL^{-1} RNasin, 0.1% DEPC, pH 7.0). The tips and tubes are RNase-free and do not require pretreatment to inactivate RNases.

2.2. Preparation of DNA-templated silver nanoclusters (DNA-AgNCs)

DNA stabilized Ag nanoclusters were synthesized according to the modifications of a literature procedure (Li et al., 2012). Briefly, 3 μM Oligo1 template or control oligonucleotide and 18 μM $AgNO_3$ were sequentially added and mixed with sodium phosphate buffer, and the reaction mixture was incubated at room temperature, in the dark, for 20 min. 18 μM $NaBH_4$ was added and the reaction mixture was incubated at room temperature, in the dark, for 14 h. Following reduction of Ag^+ ions, fluorescent DNA-AgNCs were produced with fluorescence emission at 570 nm (excitation at 465 nm).

2.3. Probe stability detection

Oligo2 template (3 μM) and 600 μM adenosine were mixed in sodium phosphate buffer for 30 min. Then the Oligo1-AgNCs complex solution was mixed with Oligo2-adenosine complex solution. After incubating at 37 °C for 5 min, the fluorescence emission spectra were recorded along with incubation time. The fluorescence emission spectra showed negligible fluorescence reduction (Fig. S1), indicating good probe stability of this probe.

2.4. Adenosine detection

Oligo2 template (3 μM) or control oligonucleotide and appropriate concentrations of adenosine were sequentially added and mixed with sodium phosphate buffer, and the reaction mixture was incubated at room temperature for 30 min. Then the prepared Oligo1-Ag NCs complex solution was mixed with Oligo2-adenosine complex solution immediately. After incubating at 37 °C for 5 min, the fluorescence emission spectra of the obtained solution were directly recorded.

2.5. Adenosine deaminase assay

The solution of the Oligo2-adenosine complex which had been prepared in Section 2.4 was treated with different concentrations ADA for 30 min. (Zhang et al., 2010, 2013) Then the prepared Oligo1-Ag NCs complex solution was mixed with ADA treated Oligo2-adenosine complex solution immediately. After incubating at 37 °C for 30 min, the fluorescence emission spectra of the obtained solution were directly recorded. It should be noted here that the employed ADA only refers to the enzymatically active protein. So, the concentration of ADA also refers to the ADA activity and enzyme unit per unit volume ($U\ L^{-1}$) is used to characterize the concentration of ADA. One unit of enzymatic activity is defined as the amount of ADA that catalyzes the conversion of 1 μM of adenosine per minute.

3. Results and discussion

3.1. The working principle

The analysis of adenosine by the kissing complexes-induced aptasensor is depicted schematically in Scheme 1. We designed our strategy based on KC24-KG51, a RNA-RNA kissing complex previously identified by Toulmé's group (Durand et al., 2014). These hairpins potentially form a 6 bp loop–loop helix that includes one GU and five GC pairs. Aptakiss was modified by adding a stem sequence and an Ag NC nucleation sequence at the 5 prime end, and aptaswitch was modified by adding a complementary

Oligo1: 5'-CCCTTAATCCCCTATAATAAATTTTTTTTTTTTTTTrUrGrCrUrCrGrGrCrCrCrCrGrCrGrArGrCrA-3'

Oligo2: 5'-TGGGGGArCrUrGrGrGrGrCrGGGAGGAATTTTTTTTTTTTTTAAATTTATTATAGGGTGGGGTGGGGTGGGG-3'

Oligo3: 5'-CCCTTAATCCCCTATAATAAATTTTTTTTTTTrUrGrCrUrCrGrGrCrCrCrCrGrCrGrArGrCrA-3'

Oligo4: 5'-TGGGGGArCrUrGrGrGrGrCrGGGAGGAATTTTTTTTTAAATTTATTATAGGGTGGGGTGGGGTGGGG-3'

Oligo5: 5'-CCCTTAATCCCCTATAATAAATTTTTTTTTTTTrUrGrCrUrCrGrGrCrCrCrCrGrCrGrArGrCrA-3'

Oligo6: 5'-TGGGGGArCrUrGrGrGrGrCrGGGAGGAATTTTTTTTTTTAAATTTATTATAGGGTGGGGTGGGGTGGGG-3'

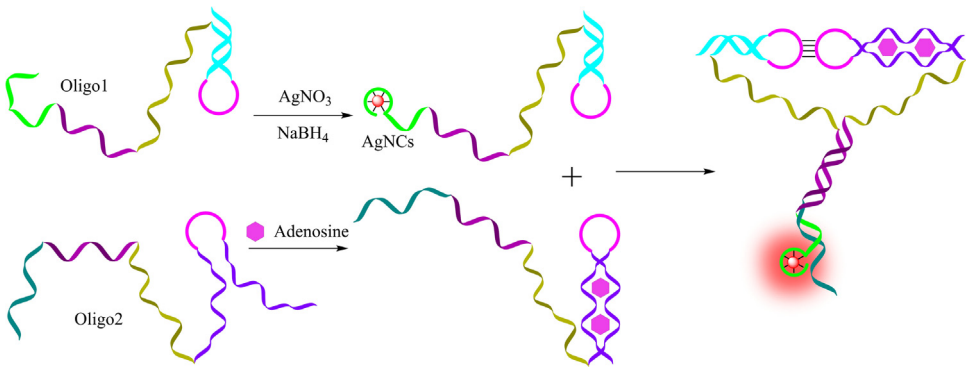
Oligo7: 5'-CCCTTAATCCCCTATAATAAATTTTTTTTTTTTTTTTrUrGrCrUrCrGrGrCrCrCrCrGrCrGrArGrCrA-3'

Oligo8: 5'-TGGGGGArCrUrGrGrGrGrCrGGGAGGAATTTTTTTTTTTTTTTAAATTTATTATAGGGTGGGGTGGGGTGGGG-3'

Oligo9: 5'-CCCTTAATCCCCTATAATAAATTTTTTTTTTTTTTTTTTrUrGrCrUrCrGrGrCrCrCrCrGrCrGrArGrCrA-3'

Oligo10: 5'-TGGGGGArCrUrGrGrGrGrCrGGGAGGAATTTTTTTTTTTTTTTTTTAAATTTATTATAGGGTGGGGTGGGGTGGGG-3'

Fig. 1. DNA oligonucleotides sequence used in this strategy. The colors of the sequences are the same as given in Scheme 1.



Scheme 1. Schematic illustration of the kissing complexes-induced aptasensor for the detection of adenosine.

stem sequence and a G-rich overhang at the 3 prime end. These complementary stem sequences are designed not to form a stable hybridization when there is no target binding. In the absence of adenosine, the aptaswitch retains the unfold state. As a result, the formation of aptaswitch-aptakiss complex is prevented, leading to the Ag NC nucleation sequence and the G-rich overhang far apart from each other which results in the observing of weak fluorescence intensity. When the target adenosine is introduced into the system, the imperfect hairpin aptamers raise against adenosine, thereby generating adenoswitch. The adenoswitch can combine with Oligo2-AgNCs to form the adenoswitch-aptakiss complex. The formation of adenoswitch-aptakiss complex then promotes hybridization between the complementary stem sequences attached to the adenoswitch and aptakiss. The hybridization brings the G-rich overhang to be close to Ag NCs, enhancing the fluorescence of Ag NCs. As a result, this assay is capable of effectively detecting adenosine.

3.2. Feasibility Study

The fluorescence signal to background ratio (F_{sig}/F_{back}) was highly sensitive to the length of poly T spacer. Adenoswitch-aptakiss complexes were formed using 400 μM adenosine and a series of probe oligo-strands by changing the length of poly T spacer from 9 to 17 bases (Fig. 1). As shown in Fig. 2A, the best fluorescence signal to background ratio was obtained when the length of poly T spacer is 13 bases. This interesting phenomenon may be caused due to the stability of hybridization part and the increase of fluorescence intensity in the absence of the target. The Oligo3 (9 bases poly T) and Oligo5 (11 bases poly T) may not hybridize with Oligo4 (9 bases poly T) and Oligo6 (11 bases poly T) effectively, respectively, which makes the Ag NC "free" in the solution, leading to the formation of AgNCs far away from G-rich overhang. Oligo7 (15 bases poly T) and Oligo9 (17 bases poly T) may hybridize with Oligo8 (15 bases poly T) and Oligo10 (17 bases poly T) effectively, respectively. However, the background in the absence of the target was increased with the prolongation of the length of poly T spacer. This phenomenon may be caused by the free hybrids between the hybridization parts (Li et al., 2012). Therefore, we chose Oligo1 and Oligo2 for the detection in this study.

To understand the time-dependent signal change of the kissing complexes-induced fluorescence aptasensor, kinetic studies have been performed by analysis of aliquots at designated time points after addition of 40 and 400 μM adenosine. As can be observed in Fig. 2B, most of the fluorescence change occurred in the first 10 min and the signals reached stable in about 30 min for both samples. Therefore, we chose the 30 min reaction time for the detection in this study. In addition, a higher adenosine concentration produced a higher final signal, which formed the basis for quantitative analysis of adenosine. The fact that each sample can reach a stable final signal suggests that the kissing complexes-induced fluorescence aptasensor can reach equilibrium.

3.3. Sensitivity of the sensing system

The as-prepared kissing complexes-induced aptasensor (KCIA) based DNA-AgNCs exhibit fluorescence emission at 465 nm with excitation at 570 nm. In the presence of adenosine, the fluorescence of the DNA-AgNCs was found to be enhanced by adenosine,

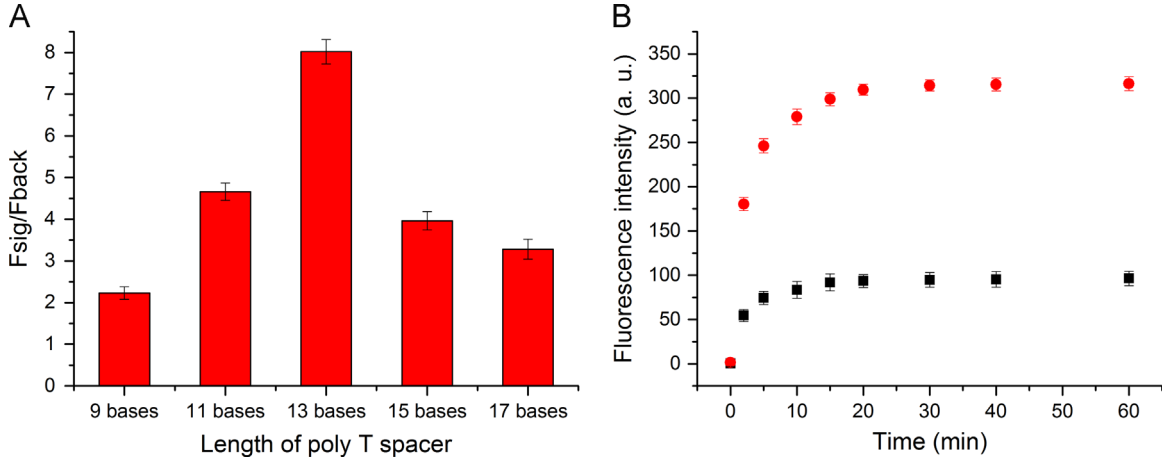


Fig. 2. (A) Effect of the length of poly T spacer on the signal-to-background ratio for detecting adenosine. The concentration of adenosine was 400 μM. (B) Signal intensity versus the time in the presence of 40 and 400 μM adenosine. All the data are taken from independent experiments with repetition for at least three times, and the presented data are the results of averaging.

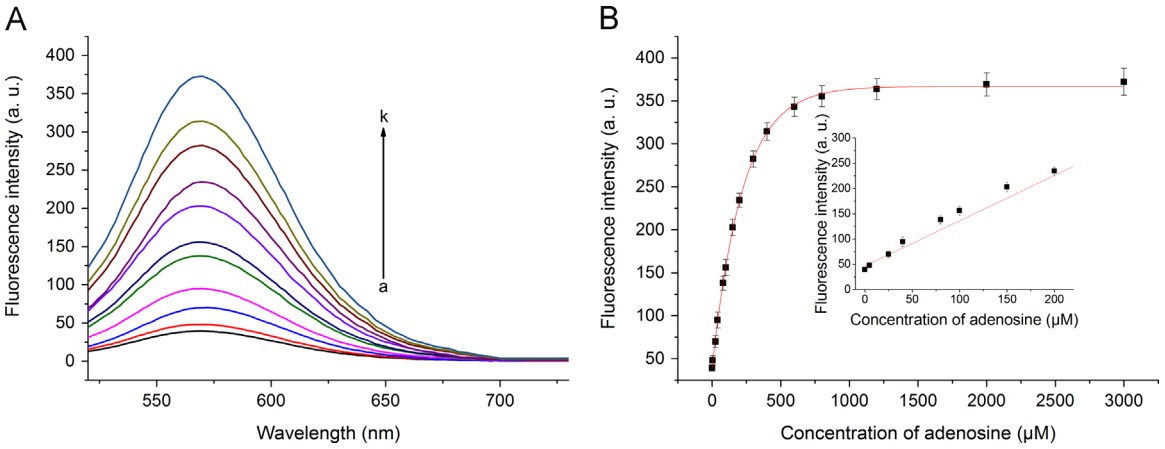


Fig. 3. (A) Emission spectra of the strategy for the assay of adenosine at different concentrations (0, 5, 25, 40, 80, 100, 150, 200, 300, 400 and 3000 μM from a to k). (B) The relationship between the fluorescence intensity increase and the concentrations of adenosine. The inset shows the linear relationship over the concentration range from 0 to 200 μM. All the data are taken from independent experiments with repetition for at least three times, and the presented data are the results of averaging.

which is attributed to the adenosine-mediated formational alteration of the adenoswitch-aptakiss complex resulting in its self-hybridization to facilitate the proximity of the G-rich overhang region and the as-prepared AgNCs templated with the NC region. Considering the appreciable changes in fluorescent properties of DNA-AgNCs toward adenosine, the potential of developing a novel fluorescent probe for determination of adenosine was assessed. To further evaluate the analytical performance of the developed method for adenosine detection based on the concept demonstrated above, the different concentrations of adenosine from one stock solution were added to the Oligo2 probes. The fluorescent probe possesses the ability to react with adenosine in a dose-response manner (Fig. 3A), which can be utilized for the quantitation of the analyte. The fluorescence intensity at 640 nm gradually increases with the increasing amount of adenosine (Fig. 3B). The fluorescence intensity versus the adenosine concentrations can be fitted to a linear regression equation from 0 to 200 μM (Fig. 3B, inset) with an equation $Y=0.89X+46.77$, where Y is the signal intensity and X is the concentration of adenosine ($R^2=0.967$). Between the amount of adenosine and the signal intensity was observed in the concentration range and the detection limit was calculated to be 2.7 μM according to the responses of the blank tests plus 3 times the standard deviation (3σ method). The proposed method had lower detection limit than some of previously reported aptasensors, as clearly shown in Table S1.

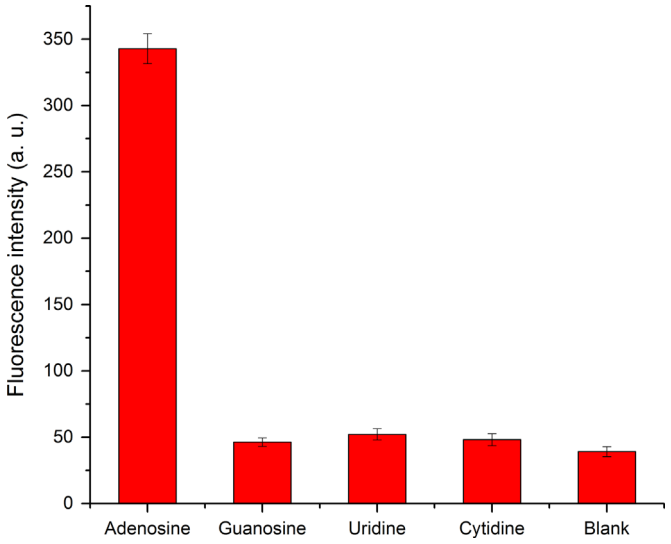


Fig. 4. Specificity test, comparing the signals from the adenosine and other non-target small molecules (guanosine, uridine and cytidine). Concentrations (adenosine, 600 μM; guanosine, 0.5 mM; and uridine and cytidine, 10 mM) were tested under the same conditions as in Fig. 3. All the data are taken from independent experiments with repetition for at least three times, and the presented data are the results of averaging.

3.4. The specificity detection

To test the selectivity, competing stimuli including Guanosine, Uridine and Cytidine at different molar levels were examined under the same conditions as in the case of adenosine (Fig. 4). It was found that adenosine results in an obvious change in the fluorescence, while there was nearly a negligible fluorescent change in the presence of other stimuli. The results demonstrated the excellent selectivity of this approach applied in adenosine detection over competing stimuli.

3.5. Detection of adenosine in complex biological system

A significant challenge for practical analyses is the ability to be applied in a complex biological system. To demonstrate the feasibility of the method in a complex biological system, adenosine spiked in human serum was evaluated with the strategy. Table S2 shows the experimental results obtained in adenosine-spiked serum samples. Adenosine concentration recoveries of 92.0–98.2% were achieved (Accuracy of intra-day in Table S2). These results showed that the interference of nuclear extracts could be overcome since the max acceptable range of recovery is 80–-120% (Cordell et al., 2014).

The measurements of intra-day and inter-day variability were utilized to determine the precision of the method. Three different concentrations (5, 40, and 100 μM) of adenosine were prepared in human serum. The relative standard deviation (RSD) was used as a measure of precision. The intra-day variability was examined within one day ($n=5$) and inter-day variability was determined in quadruplication on 3 separate days. The intra-day precision is shown in Table S2 where the RSDs ranged from 3.2% to 4.9%. The inter-day precision was determined from twelve determinations over 3 separate days for each concentration and the results were in the range of 3.5% to 4.2%. These results showed that this strategy has a high precision.

3.6. The assay of ADA

ADA is an enzyme involved in purine metabolism. ADA irreversibly deaminates adenosine, converting it to the inosine by the

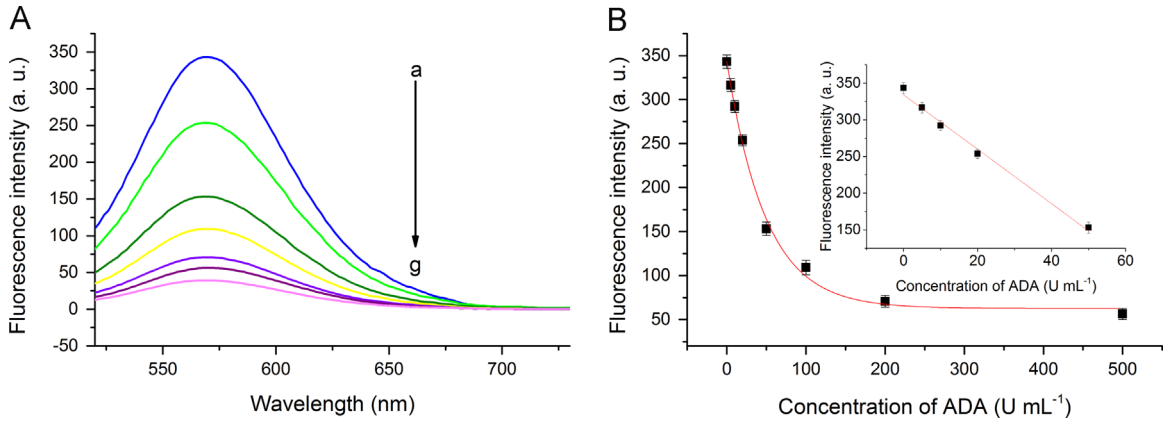


Fig. 5. (A) Emission spectra of the strategy for the assay of ADA at different concentrations (0, 20, 50, 100, 200, and 500 U mL^{-1} from a to f, and Oligo1-AgNCs/Oligo2 complex (g)). (B) The relationship between the fluorescence intensity and the concentrations of ADA. The inset shows the linear relationship over the concentration range from 0 to 50 U mL^{-1}. All the data are taken from independent experiments with repetition for at least three times, and the presented data are the results of averaging.

substitution of the amino group for a hydroxyl group (Zhang et al., 2010). ADA is a 41-KDa zinc-dependent enzyme in the purine salvage pathway found ubiquitously in all human tissues in predominantly cytosolic and membrane-bound isoforms especially in lymphoid system (Zhang et al., 2012). On the other hand, ADA deficiency is an autosomal recessive metabolic disorder that causes immunodeficiency (Aiuti et al., 2009). Therefore, the assay for the activity of ADA is important and of general interest toward biochemical research, diagnosis of ADA related diseases. Because ADA can catalyze the conversion of adenosine to inosine at the state of binding with the aptamer, then the inosine will not bind with the aptamer anymore. Thus the target-induced conformational alteration of aptamer can be recovered upon the transformation of adenosine into inosine. Then we further investigated the feasibility of the assay for ADA on the basis of the above KCIA based Oligo-AgNCs biosensor in the Oligo2-adenosine state (600 μM adenosine added). Different amounts of ADA from one stock solution were added to the above KCIA based Oligo-AgNCs biosensor, a gradual fluorescence decrease in the DNA-AgNCs molecular beacon was observed (Fig. 5A). The fluorescent decrease of DNA-AgNCs molecular beacon can be attributed to the recovery of conformational alteration of the aptamer region by ADA, which weakens the proximity of the G-rich overhang region and the as-prepared AgNCs templated with the NC region. Fig. 5B presents the relationship between the peak currents and the quantity of ADA. The fitting equation of the curve shown in Fig. 5B is $y = 62.67+280.91*e^{-0.020X}$. It is half of a hyperbola, which is a typical fitting mode for an enzyme assay. Also, a detection limit of 4 U mL^{-1} can be obtained according to the responses of the blank tests plus 3 times the standard deviation (3σ method). The fluorescence intensity versus the ADA concentrations can be fitted to a linear regression equation from 0 to 50 U mL^{-1} (Fig. 5B, inset) with an equation $Y=-3.71X+333.94$, where Y is the signal intensity and X is the concentration of adenosine ($R^2=0.989$). Thus, the results of the assay of the enzymatic activity by this KCIA based biosensor are demonstrated to work well.

4. Conclusions

In summary, we have successfully demonstrated that the kissing complexes-induced aptasensor (KCIA) based biosensor can be used as a fluorescent biosensor for label-free detection of adenosine and for further monitoring the activity of adenosine deaminase using the guanine-rich (G-rich) DNA sequence as a signal transducer, which exhibits intrinsic attractive properties and expands the application of AgNCs. This is a new concept for aptamer based biosensors, and opens an opportunity for design of more novel biosensors based on the kissing complexes-induced strategy.

Acknowledgment

This work is supported by the Social Development Fund of Jiangsu Province (BE2013614), the Grants from National Natural Science Foundation of China (81300787), the Natural Science Foundation of Jiangsu Province (BK2011168, BK2012105, BK20041103), and Technology Infrastructure Plan of Jiangsu Province-Technology Public Service Platform (BM2012066).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2015.11.038.

References

Aiuti, A., Cattaneo, F., Galimberti, S., Benninghoff, U., Cassani, B., Callegaro, L., Scaramuzza, S., Andolfi, G., Mirolo, M., Brigida, I., Tabucchi, A., Carlucci, F., Eibl, M., Aker, M., Slavin, S., Al-Mousa, H., Al Ghonaium, A., Ferster, A., Duppenthaler, A., Notarangelo, L., Wintergerst, U., Buckley, R.H., Bregni, M., Marktel, S., Valsecchi, M.G., Rossi, P., Ciceri, F., Miniero, R., Bordignon, C., Roncarolo, M.-G., 2009. Gene therapy for immunodeficiency due to adenosine deaminase deficiency. N. Engl. J. Med. 360 (5), 447–458.

Brunel, C., Marquet, R., Romby, P., Ehresmann, C., 2002. RNA loop–loop interactions as dynamic functional motifs. Biochimie 84 (9), 925–944.

Cordell, R., White, I., Monks, P., 2014. Validation of an assay for the determination of levoglucosan and associated monosaccharide anhydrides for the quantification of wood smoke in atmospheric aerosol. Anal. Bioanal. Chem. 406 (22), 5283–5292.

Ducongé, F., Eacute, Eacute, R.I.C., Toulmé, J.-J., 1999. In vitro selection identifies key determinants for loop–loop interactions: RNA aptamers selective for the TAR RNA element of HIV-1. RNA 5 (12), 1605–1614.

Durand, G., Lisi, S., Ravelet, C., Dausse, E., Peyrin, E., Toulmé, J.-J., 2014. Riboswitches based on kissing complexes for the detection of small ligands. Angew. Chem. Int. Ed. 53 (27), 6942–6945.

Ennifar, E., Walter, P., Ehresmann, B., Ehresmann, C., Dumas, P., 2001. Crystal structures of coaxially stacked kissing complexes of the HIV-1 RNA dimerization initiation site. Nat. Struct. Mol. Biol. 8 (12), 1064–1068.

Huang, Z., Pu, F., Lin, Y., Ren, J., Qu, X., 2011. Modulating DNA-templated silver nanoclusters for fluorescence turn-on detection of thiol compounds. Chem. Commun. 47 (12), 3487–3489.

Huang, Z., Tao, Y., Pu, F., Ren, J., Qu, X., 2012. Versatile logic devices based on programmable dna-regulated silver-nanocluster signal transducers. Chem. Eur. J. 18 (21), 6663–6669.

Li, J., Zhong, X., Zhang, H., Le, X.C., Zhu, J.-J., 2012. Binding-induced fluorescence turn-on assay using aptamer-functionalized silver nanocluster DNA probes. Anal. Chem. 84 (12), 5170–5174.

Liu, Y.-Q., Zhang, M., Yin, B.-C., Ye, B.-C., 2012. Attomolar ultrasensitive MicroRNA

detection by DNA-scaffolded silver-nanocluster probe based on isothermal amplification. Anal. Chem. 84 (12), 5165–5169.
Mandal, M., Breaker, R.R., 2004. Gene regulation by riboswitches. Nat. Rev. Mol. Cell. Biol. 5 (6), 451–463.
Sharma, J., Rocha, R.C., Phipps, M.L., Yeh, H.-C., Balatsky, K.A., Vu, D.M., Shreve, A.P., Werner, J.H., Martinez, J.S., 2012. A DNA-templated fluorescent silver nanocluster with enhanced stability. Nanoscale 4 (14), 4107–4110.
Yang, S.W., Vosch, T., 2011. Rapid detection of MicroRNA by a silver nanocluster DNA probe. Anal. Chem. 83 (18), 6935–6939.
Yeh, H.-C., Sharma, J., Shih, I.-M., Vu, D.M., Martinez, J.S., Werner, J.H., 2012. A fluorescence light-up Ag nanocluster probe that discriminates single-nucleotide variants by emission color. J. Am. Chem. Soc. 134 (28), 11550–11558.
Zhang, K., Yang, Q., Zhang, J., Fu, L., Zhou, Y., Wu, B., Xie, M., Huang, B., 2013. An enzyme substrate binding aptamer complex based time-resolved fluorescence sensor for the adenosine deaminasedetection. Biosens. Bioelectron. 42 (0), 87–92.
Zhang, K., Wang, K., Zhu, X., Gao, Y., Xie, M., 2014a. Rational design of signal-on biosensors by using photoinduced electron transfer between Ag nanoclusters and split G-quadruplex halves-hemin complexes. Chem. Commun. 50 (91), 14221–14224.
Zhang, K., Wang, K., Zhu, X., Zhang, J., Xu, L., Huang, B., Xie, M., 2014b. Label-free and ultrasensitive fluorescence detection of cocaine based on a strategy that utilizes DNA-templated silver nanoclusters and the nicking endonuclease-assisted signal amplification method. Chem. Commun. 50 (2), 180–182.
Zhang, K., Zhu, X., Wang, J., Xu, L., Li, G., 2010. Strategy to fabricate an electrochemical aptasensor: application to the assay of adenosine deaminase activity. Anal. Chem. 82 (8), 3207–3211.
Zhang, M., Guo, S.-M., Li, Y.-R., Zuo, P., Ye, B.-C., 2012. A label-free fluorescent molecular beacon based on DNA-templated silver nanoclusters for detection of adenosine and adenosine deaminase. Chem. Commun. 48 (44), 5488–5490.