Nanomedicine Journal

Received: Jun. 26, 2013; Accepted: Jul. 29, 2013
Vol. 1, No. 2, Winter 2014, page 100-106

Online ISSN 2322-5904
http://nmj.mums.ac.ir

Original Research

A novel label-free cocaine assay based on aptamer-wrapped single-walled carbon nanotubes

Sahar Taghavi[1,2#], Sara Ayatollahi[1,2#], Mona Alibolandi[3], Parirokh Lavaee[4], Mohammad Ramezani[1,5*], Khalil Abnous[1,2*]

[1] Pharmaceutical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[2] Department of Pharmaceutical Biotechnology, Mashhad University of Medical Sciences, Mashhad, Iran
[3]Biotechnology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[4]Department of Chemistry, Ferdowsi University of Mashhad, Mashhad, Iran
[5] Nanotechnology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Abstract

Objective(s): This paper describes a selective and sensitive biosensor based on the dissolution and aggregation of aptamer wrapped single-walled carbon nanotubes. We report on the direct detection of aptamer–cocaine interactions, namely between a DNA aptamer and cocaine molecules based on near-infrared absorption at λ_{807}.
Materials and Methods: First a DNA aptamer recognizing cocaine was non-covalently immobilized on the surface of single walled carbon nanotubes and consequently dissolution of SWNTs was occurred. Vis-NIR absorption (A_{807nm}) of dispersed, soluble aptamer-SWNTs hybrid, before and after incubation with cocaine was measured using a CECIL9000 spectrophotometer.
Results: This carbon nanotube setup enabled the reliable monitoring of the interaction of cocaine with its cognate aptamer by aggregation of SWNTs in the presence of cocaine.
Disscusion: This assay system provides a mean for the label-free, concentration-dependent, and selective detection of cocaine with an observed detection limit of 49.5 nM.

Keywords: Aptamer, Cocaine, Near infrared absorption, Single-walled carbon nanotube

***Corresponding authors:** Khalil Abnous (Ph.D.), Mohammad Ramezani (Ph.D.), Pharmaceutical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.*
Email: abnouskh@mums.ac.ir or Email: ramezanim@mums.ac.ir
#Sahar Taghavi and Sara Ayatollahi contributed equally to this work.

Introduction

Simple and sensitive detection of cocaine is very important to fight against the illegal usage of this compound. However, this has remained as a bottleneck in cocaine identification and quantification until the development of specific aptamers against it (1-9). Nowadays aptamers are of increasing interest in being used as recognition element within sensors for small molecules (10-13). Aptamers exhibit several advantages such as high affinity and specificity with respect to target recognition. Moreover, in comparison with antibodies, they are resistant to cycles of denaturation-renaturation, and are less prone to degradation. Furthermore, they can be chemically synthesized and, thus, adopted to develop biosensors.

So far several nucleic acid aptamer-based bisensors for cocaine detection have been developed. The cocaine quantification approach in developed aptameric biosensor was mainly based on colorimetric (1-4), electrochemical (5) and fluorescence techniques (6-9).

However, these reported aptameric sensors either exhibit relatively hard sample preparation, inadequate sensitivity, or high sample consumption. Consequently, the development of alternative simple and sensitive aptameric sensors is highly desirable.

Single-walled carbon nanotubes (SWNT) exhibit excellent optical properties, including Raman light scattering, near-infrared (NIR) fluorescence and NIR absorption (14-16).

The optical properties of SWNT make them suitable to be employed for the development of biological sensors due to their NIR photo luminescence/absorption where absorption and auto-fluorescence by tissues, biological fluids and water are minimized (17, 18). Although SWNTs have near-infrared (NIR) absorption and photoluminescence properties which make them interesting as biosensor but their photoluminescence is observed only when they are individually dispersed (19).

CNTs are extremely hydrophobic and are insoluble in water.

Two methods have been employed to modify the hydrophobic surface of CNTs to increase their solubility.

The first was to disperse carbon nanotubes in aqueous solution by covalent association of water soluble materials to the CNT surface, and the second approach was used the noncovalent association of materials to the CNT surface (20-26).

In both approaches, various organic or biological materials have been utilized successfully including oligonucleotides (27), peptides (28) and proteins (29).

Meanwhile, covalent association of materials to the surface of CNTs is appropriate for many applications, although one important disadvantage is that the covalent association introduces defects in the surface of the CNTs that often interfere with the electronic and optical properties. Thus non-covalent attachment of materials to CNTs is more appropriate for increasing their solubility.

In the case of aptamer-wrapped carbon nanotubes, the structural change in nucleic acid aptamers upon interaction with target molecules sufficiently perturbs the electronic structure of SWNTs such that the change can be detected optically (30).

In current study, aptamers were immobilized non-covalently on the surface of SWNTs via π-stacking interactions between the nucleotide bases and the SWNT sidewalls and the specific interaction of aptamers wrapped on the sidewall of SWNTs with cocaine was detected via near-infrared absorption (Figure 1).

Materials and Methods

General

All chemicals and reagents were purchased from Sigma Aldrich (Germany) with the highest quality available.

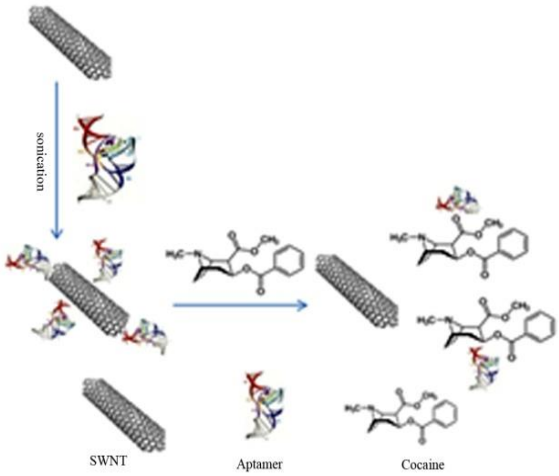

Figure 1. Schematic illustration of aptameric sensor for cocaine sensing. Cocaine aptamer was noncovalently attached to SWCTs. Upon cocaine interaction, aptamer was unwrapped from SWNTs and the changes in carbon nanotubes solubility were detected.

Synthesis of aptamer

An anti cocaine ssDNA aptamer (5' GACAAGGAAAATCCTTCAATGAAGT GGGTC 3') reported by Cekan and colleagues (31) was employed as bioreceptor.

Cocaine ssDNA aptamer was synthesized using standard phosphoramidate chemistry and purified by polyacrylamide denaturating gel electrophoresis to remove the truncated DNA fragments produced in the chemical synthesis.

Noncovalent conjugation of aptamer on the surface of SWNTs

At first stage, SWNTs (0.5 mg) and DNA aptamer (133 μM) in 2 ml of Tris-HCl buffer (10 mM, pH 7.4) was sonicated for 2 h. Then mixture was centrifuged at 15000 **g** for 2 h and pellets containing impurities, aggregates, and bundles of nanotubes were discarded.

Then the supernatant was colle-cted and underwent an additional centri-fugation round and finally supernatant con-taining aptamer-SWNT complexes was collected (32).

Quantitative determination of cocaine

Different concentrations of cocaine (0, 50, 100, 150 and 200 nM) was added to aptamer-SWNT complexes and incubated for 24 h at 37°C.

Vis-NIR absorption (A_{807nm}) of dispersed, soluble aptamer-SWNTs hybrid, before and after incubation with cocaine was measured using a CECIL9000 spectrophotometer (Cecil Company, UK) (32).

Results

Purification and czharacterization of synth-esized aptamer

Synthesized DNA aptamer was purified from polyacrylamide denaturating gel (7 M urea) (Figure 2).

A_{260}/A_{280} of synthesized DNA-aptamer and its concentration was 1.83 and 1250 ng/μl, respectively.

Secondary structure of synthesized cocaine aptamer was predicted by Mfold tool (33). As shown in Figure 3, the folded cocaine aptamer contains one hairpin-loop motif and the ΔG was - 2.45.

Figure 2. Denaturing urea polyacrylamide gel electrophoresis was used to separate and purify synthesized single-stranded DNA aptamer of cocaine. Denaturing PAGE was placed on a fluorescence TLC plate. Aptamers and other truncated oligonucleotides appeared as black bands. Upper band were cut and aptamers were purified by several thaw-freeze cycles.

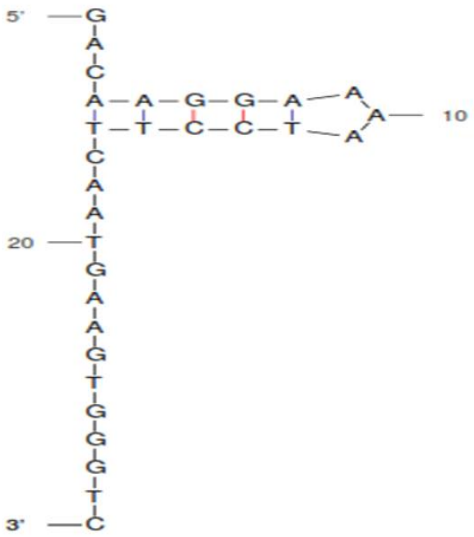

Figure 3. Secondary structure of cocaine ssDNA aptamer as predicted by Mfold (ΔG = - 2.45).

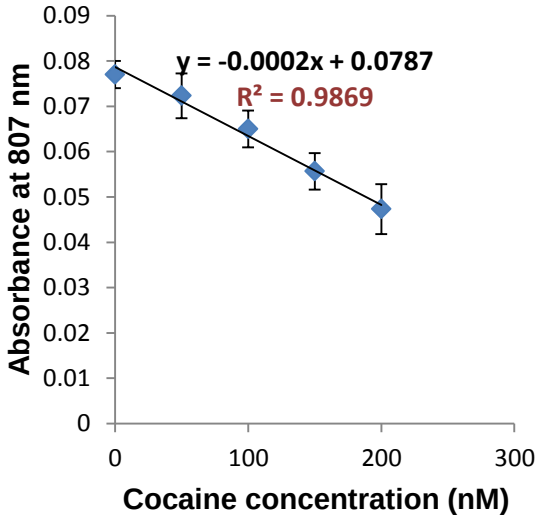

Figure 4. Standard curve for determination of cocaine using SWNT-based aptameric biosensor.

Cocaine determination

Aptamer-SWNT complex was incubated with different concentrations (0-200 nM) of cocaine and the standard curve was plotted at λ_{807} nm.

After adding cocaine into the SWNT–aptamer solution, the UV-Vis-NIR absorption at 807 nm decreased as shown in Figure 4 indicating that the SWNT gradually lost its aptamer-induced dispersion stability and tended to aggregate.

Coefficient of determination (r^2) was meas-ured to be 0.987 which demonstrated good linearity between aptamer-SWCTs complex absorption at λ_{807} versus cocaine concen-tration (Figure 4).

These results clearly indicated that the aggregation fashion was obviously dependent on the concentration of cocaine. The limit of detection (LOD) and limit of quantification (LOQ) was calculated according to equations 1 and 2 where "SD" is standard deviation of the response and "S" is slope of the calibration curve:

$$LOD=3.3(SD/S) \quad (1)$$

$$LOD_{(cocaine)}=3.3(0.003/0.0002)=49.5 \text{ nM}$$

$$IOQ=10(SD/S) \quad (2)$$

$$LOQ_{(cocaine)}=10(0.003/0.0002)=150 \text{ nM}$$

Discussion

SWNTs contain extremely delocalized π electrons; consequently the surface of SWNTs can be easily functionalized through π-π interactions with compounds that possess a π-electron-rich structure such as nucleic acid aptamers.

Non-covalent association of nucleic acid aptamers to the CNT surface increases its solubility (34).

Previously it was proved that SWNT–aptamer hybrid showed the same semi-conductive band gap between 800 nm to 1350 nm and the electronic properties of SWNT were maintained intact after the DNA wrapping (19).

Furthermore, the solublized aptamer-wrapped SWNTs are well dispersed, mostly as individual tubes.

After addition of target molecules, aptamers were unwrapped and the amount of soluble SWNTs decreased which could be detected optically.

It is worth mentioning that the main shortcoming using an aptameric sensor for cocaine detection and quantification is its low detection sensitivity which is limited by their relatively low association constant (35).

The best reported detection limits are 10

μM, 5 μM and 0.5 μM of cocaine for various assay techniques including electrochemical method (5), aptamer-based enzymatic assay (6), bulk fluorescence (7) and aptamer-based colorimetric probes (4).

In this regards, Zhou et al. fabricated FITC-labeled aptamer sensor for detection of cocaine. This sensor showed a detection limit of 0.2 μM (36).

The detection limit of our cocaine-aptameric sensor was more desirable than those reported before, with neither complicated sample preparation nor large analyte volume.

It should be noted that most laboratory utilized mass spectral analysis to detect cocaine, which requires the use of stable isotopes as internal standards, very time consuming sample preparation and application of expensive instruments (37, 38).

In contrast, our non-labeled aptameric sensor took advantage of a simple sample preparation, with potential to be applied for simple and low cost detection of cocaine.

Conclusion

A novel optical cocaine sensor was designed using the reversible dissolution and aggregation of SWNTs based on interaction of cocaine with its specific ssDNA aptamer. Our results revealed that the aptameric sensor was comparatively sensitive with a low detection limit.

Prepared cocaine aptameric sensor works based on optically detection of solubility reduction of aptamer-SWNT hybrids upon binding of aptamer to cocaine.

Thus, developed assay technique did not require either chemical functionalization or labeling of SWNTs and DNA owing to the exclusive NIR absorption at 807 nm of dispersed SWNTs.

With the advances in the development of varieties of aptamers for small molecules, this aptamer-wrapped single-walled carbon nanotube sensor might find wide application in forensic analysis and clinical diagnostics.

Acknowledgments

This study was a part of a thesis of S. Taghavi and it has been supported financially by Mashhad University of Medical Sciences.

References

1. Liu J, Lu Y. Non-base pairing DNA provides a new dimension for controlling aptamer-linked nanoparticles and sensors. J Am Chem Soc. 2007; 129: 8634–8643.
2. Liu J, Lu Y. Rational design of "turn-on" allosteric DNAzyme catalytic beacons for aqueous mercury ions with ultrahigh sensitivity and selectivity. Angew Chem Int Ed. 2006; 45: 90–94.
3. Zhang J, Wang L, Pan D, Song S, Boey FYC, Zhang H, Fan C. Visual cocaine detection with gold nanoparticles and rationally engineered aptamer structures. Small. 2008; 4: 1196–1200.
4. Stojanovic MN, Landry DW. Aptamer-based colorimetric probe for cocaine. J Am Chem Soc. 2002; 124: 9678–9679.
5. Baker BR, Lai RY, Wood MS, Doctor EH, Heeger AJ, Plaxco KW. An electronic, aptamer-based small-molecule sensor for the rapid, label-free detection of cocaine in adulterated samples and biological fluids. J Am Chem Soc. 2006; 128: 3138–3139.
6. Stojanovic MN, Parada P, de, Landry DW. Aptamer-based folding fluorescent sensor for cocaine. J Am Chem Soc. 2001; 123: 4928–4931.
7. Stojanovic MN, de Parada P, Landry DW. Fluorescent sensors based on aptamer self-assembly. J Am Chem Soc. 2000; 122: 11547–11548.
8. Shlyahovsky B, Di L, Weizmann Y, Nowarski R, Kotler M, Willner I. Spotlighting of cocaine by an autonomous aptamer-based machine. J Am Chem Soc. 2007; 12: 3814–3815.
9. Liu J, Lee JH, Lu Y. Quantum dot encoding of aptamer-linked nanostructures for one-pot simultaneous detection of multiple analytes. Anal Chem. 2007; 79: 4120–4125.
10. Jhaveri SD, Kirby R, Conrad R, Maglott EJ, Bowser M, Kennedy RT, Glick G, Ellington AD. Designed signaling aptamers that transduce molecular recognition to changes in fluorescence

intensity. J Am Chem Soc. 2000; 122: 2469–2473.
11. Liss M, Petersen B, Wolfe H, Prohaska E. An aptamer-based quartz crystal protein biosensor. Anal Chem. 2002; 74: 4488–4495.
12. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science. 1990; 249: 505–510.
13. Nutiu R, Li Y. Structure-switching signaling aptamers. J Am Chem Soc. 2003; 125: 4771–4778.
14. Avouris, P. Molecular electronics with carbon nanotubes. Acc Chem Res. 2002; 35: 1026-1034.
15. Bachilo, SM, Strano MS, Kittrell C, Hauge RH, Smalley RE, Weisman RB. Structure-assigned optical spectra of single-walled carbon nanotubes. Science. 2002; 298: 2361-2366.
16. Barone PW, Baik S, Heller DA, Strano MS. Near-infrared optical sensors based on single-walled carbon nanotubes. Nat Mater. 2005; 4: 86-92.
17. Heller DA, Baik S, Eurell TE, Strano MS. Single-walled carbon nanotube spectroscopy in live cells: towards long-term labels and optical sensors. Adv Mater. 2005; 17: 2793-2799.
18. Wray S, Cope M, Delpy DT, Wyatt JS, Reynolds EOR. Characterization of the near infrared absorption spectra of cytochrome aa3 and haemoglobin for the non-invasive monitoring of cerebral oxygenation. Biochim Biophys Acta. 1988; 933: 184-192.
19. Chen H, Yu C, Jiang C, Zhang S, Liu B, Kong J. A novel near-infrared protein assay based on the dissolution and aggregation of aptamer-wrapped single-walled carbon nanotubes. Chem Commun. 2009; 33: 5006-8.
20. Tasis D, Tagmatarchis N, Bianco A, Prato M: Chemistry of carbon nanotubes. Chem Rev. 2006; 106: 1105-1136.
21. Lin Y, Taylor S, Li H, Fernando KAS, Qu L, Wang W, Gu L, Zhou B, Sun YP: Advances toward bioapplications of carbon nanotubes. J Mater Chem. 2004; 14: 527-541.
22. Bahr JL, Tour JM: Covalent chemistry of single-wall carbon nanotubes. J Mater Chem. 2002; 12: 1952-1958.
23. Banerjee S, Hemraj-Benny T, Wong SS: Covalent surface chemistry of single-walled carbon nanotubes. Adv Mater (Weinheim, Germany). 2005; 17: 17-29.
24. Tasis D, Tagmatarchis N, Georgakilas V, Prato M. Soluble carbon nanotubes. Chem Eur J. 2003; 9: 4000-4008.
25. Sun YP, Fu K, Lin Y, Huang W: Functionalized carbon nanotubes: properties and applications. Acc Chem Res. 2002; 35: 1096-1104.
26. Niyogi S, Hamon MA, Hu H, Zhao B, Bhowmik P, Sen R, Itkis ME, Haddon RC: Chemistry of Single-Walled Carbon Nanotubes. Acc Chem Res. 2002; 35: 1105-1113.
27. Chou SG, Plentz F, Jiang J, Saito R, Nezich D, Ribeiro HB, Jorio A, Pimenta MA, Samsonidze GG, Santos AP, Zheng M, Onoa GB, Semke ED, Dresselhaus G, Dresselhaus MS: Phonon-Assisted Excitonic Recombination Channels Observed in DNA-Wrapped Carbon Nanotubes Using Photoluminescence Spectroscopy. Phys Rev Lett. 2005; 94: 127402/1-127402/4.
28. Arnold MS, Guler MO, Hersam MC, Stupp SI: Encapsulation of Carbon Nanotubes by Self-Assembling Peptide Amphiphiles. Langmuir. 2005; 21: 4705-4709.
29. Kam NWS, Jessop TC, Wender PA, Dai H: Nanotube molecular transporters: internalization of carbon nanotube-protein conjugates into mammalian cells. J Am Chem Soc. 2004; 126: 6850-6851.
30. Heller DA, Jeng ES, Yeung TK, Martinez BM, Moll AE, Gastala JB, Strano MS: Optical Detection of DNA Conformational Poly-morphism on Single-Walled Carbon Nanotubes. Science. 2006; 311: 508-511.
31. Cekan P, Jonsson EÖ, Sigurdsson ST. Folding of the cocaine aptamer studied by EPR and fluorescence spectroscopies using the bifunctional spectroscopic probe C. Nucleic Acids Res. 2009; 37(12): 3990-3995.
32. Chen H, Yu C, Jiang C, Zhang S, Liu B, Kong J. A novel near-infrared protein assay based on the dissolution and aggregation of aptamer-wrapped single-walled carbon nanotubes. Chem Commun. 2009; 33: 5006-5008.
33. Zuker M. Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res. 2003; 31(13): 3406-3415.
34. Taghdisi SM, Lavaee P, Ramezani M, Abnous K. Reversible targeting and controlled release delivery of daunorubicin to cancer cells by aptamer-wrapped carbon nanotubes. Eur J Pharm Biopharm. 2011; 77(2): 200-206.

35. Hamula CLA, Guthrie JW, Zhang H, Li XF, Le XC. Selection and analytical applications of aptamers. Trends Anal Chem. 2006; 7: 681–691.
36. Zhou J, Ellis AV, Kobus H, Voelcker NH. Aptamer sensor for cocaine using minor groove binder based energy transfer. Anal chim acta. 2012; 719: 76-81.
37. Cone EJ, Hillsgrove M, Darwin WD. Simultaneous measurement of cocaine, cocaethylene, their metabolites, and "crack" pyrolysis products by gas chromatography-mass spectrometry. Clinic Chem. 1994; 40: 1299–1305.
38. Chinn DM, Crouch DJ, Peat MA, Finkle BS, Jennison TA. Gas chromatography-chemical ionization mass spectrometry of cocaine and its metabolites in biological fluids. J Anal Toxicol. 1980; 4: 37–42.