doi:10.1246/cl.2010.454
Published on the web March 31, 2010

Electronic Aptamer-based Biosensor for Multiprotein Analytes on a Single Paltform

Eriko Hayashi, Tadao Takada, Mitsunobu Nakamura, and Kazushige Yamana*
Department of Materials Science and Chemistry, University of Hyogo, 2167 Shosha, Himeji 671-2201

(Received February 2, 2010; CL-100104; E-mail: yamana@eng.u-hyogo.ac.jp)

We describe an approach to the development of a new electronic aptamer-based biosensor for multiprotein targets on a single platform. The multiple protein biosensing involves the co-adsorption of several thiol-terminated capture DNA sequences on a gold surface, along with the hybridization of redox-tagged aptamer to each corresponding capture sequences on the electrode, addition of target proteins, and monitoring aptamer-strand release through electrochemical measurement.

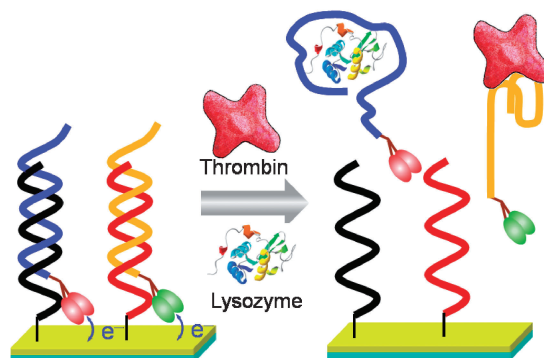
Aptamers are single-strand nucleic acid sequences that can be obtained by in vitro selection (SELEX). Because of their high affinity and selectivity for a wide range of target ligands and their ease of synthesis and chemical modification, the biosensor applications of aptamers hold great promise.[1–3] A number of examples of optical and electrochemical aptamer-based biosensors have been reported.[1–5] However, many of these aptamer biosensors are designed to detect a single analyte, although the detection of multi-analytes such as protein targets is highly desirable in medicinal diagnostics and proteomics.[1–3,6–12]

The present report describes a simple and easy approach to the development of an electrochemical apatamer-based biosensor (E-aptasensor) for the detection of multiprotein targets on a single platform.

Our group and others have demonstrated a target-induced strand release (TISR) strategy for the development of E-aptasensors.[13–15] The features of TISR strategy are that 1) redox-tagged aptamer sequences are chemically synthesized by using a redox-tagged phosphoramidite derivative. 2) The aptasensors can be fabricated by hybridization of redox-aptamers to capture DNA sequences that are prebound onto a gold electrode. 3) The presence of target can be determined from signal suppression due to the release of redox-tagged aptamer upon binding to the target. With this strategy, label-free, rapid, selective, and sensitive sensors particularly for large proteins are produced. The features of the E-aptasensors prompted us to develop a new platform for an electrochemical detection scheme for multiprotein targets.

As shown in Scheme 1, the new multiple protein biosensing involves the coadsorption of several thiol-terminated capture DNA sequences on a gold surface, along with the hybridization of redox-tagged aptamers to the corresponding capture sequences at the electrode, addition of target proteins, and monitoring the aptamer-strand release through electrochemical measurement. Our single-platform aptasensor based on TISR approach offers simultaneous detection of multiple target proteins.

To test the validity of our approach, we used two different aptamers[1–3] [Thrombin aptamer (27-mer) and Lysozyme aptamer (42-mer)], which were labeled at the 5′-terminus by ferrocene (**X**) and anthraquinone (**Y**), respectively. With this labeling, each aptamer will exhibit a distinct electrochemical response. We decided to use Capture DNA-T that has complementary sequence (21-mer) to the thrombin aptamer and Capture DNA-L (20-mer) to the lysozyme aptamer as a capture strand, because of the following observations. Judging from the T_m values as shown in Table 1, Thrombin aptamer–Capture DNA-T and Lysozyme aptamer–Capture DNA-L duplexes are sufficiently stable at room temperature. By using the redox-modified duplex

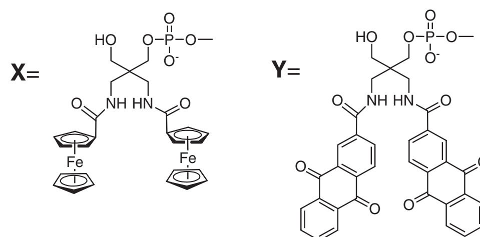


Scheme 1. Schematic representation of multiprotein sensing based on redox-modified aptamer–capture DNA duplex on a single electrode.

Table 1. Sequences and T_m values for redox-modified aptamer−capture DNA duplex

Sequences	Duplex $T_m{}^a$/°C
Thrombin aptamer	
5′-**X**CCA TCT CCA CTT GGT TGG TGT GGT TGG	
Capture DNA-T	
5′-ACA CCA ACC AAG TGG AGA TGG TTT T–$(CH_2)_3SS(CH_2)_3OH$	65.8[a]
Lysozyme aptamer	
5′-**Y**ATC TAC GAA TTC ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG	
Capture DNA-L	
5′-GCC CTG ATG AAT TCG TAG ATT TTT T–$(CH_2)_3SS(CH_2)_3OH$	63.7[b]

[a]T_m values were determined from UV-melting curves obtained for duplex (2 μM) in 0.1 M NaCl and 0.01 M sodium phosphate (pH 7).



© 2010 The Chemical Society of Japan

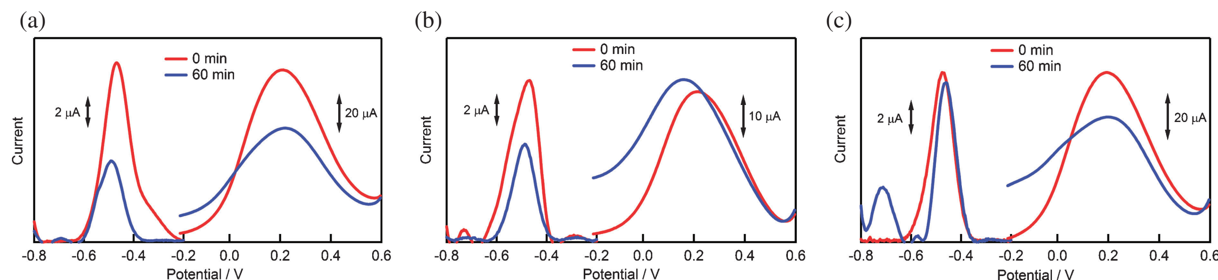


Figure 1. Differential pulse voltammograms (DPV) of electronic aptamer-based biosensor (E-aptasensor) for multiprotein targets, thrombin and lysozyme. DPV measurements were carried out at room temperature in a degassed buffer (2 mL) containing 300 mM NaCl, 50 mM $MgCl_2$, and 20 mM Tris-HCl (pH 7.6). Panel (a): DPV of E-aptasensor before and after addition of thrombin (100 nmol) and lysozyme (100 nmol). Panel (b): DPV of E-aptasensor before and after addition of lysozyme (100 nmol). Panel (c): DPV of E-aptasensor before and after addition of thrombin (100 nmol).

with a disulfide linker at the one terminus, we first prepared and tested E-aptasensor that can sense a single protein analyte.[13] The single targeting sensors exhibited similar performance in protein sensing (Supporting Information).[16] 10% decrease in the presence of 10 nmol of protein and 40–50% decrease for 100 nmol protein were observed. With the thrombin sensor, 500 nmol of thrombin analyte gave 65% decrease of the electrochemical signal.

A single platform multiprotein sensor was then fabricated as follows: A mixture of Capture DNA-T (5 μM) and DNA-L (5 μM) in 50 μL of 200 mM Tris-HCl (pH 7.4) containing 0.1 mM tris(2-carboxyethyl)phosphine hydrochloride was deposited onto a gold electrode surface (0.5 cm^2) at room temperature for 16 h. The modified surface was then treated with 2.5 mM of 3-mercaptopropanol in 10 mM Tris-HCl (pH 7.4) for 1 h. The subsequent hybridization was carried out with a mixture of Thrombin and Lysozyme aptamer (10 μM each) in 50 μL of 10 mM sodium phosphate (pH 7) containing 100 mM NaCl and 10 mM $MgCl_2$ at room temperature for 3 h.[17] DPV measurements[18] were carried out at room temperature in a degassed buffer (2 mL) containing 300 mM NaCl, 50 mM $MgCl_2$, and 20 mM Tris-HCl (pH 7.6) by using a three-electrode system consisting of a DNA-modified working electrode (0.5 cm^2), Pt-wire as a counter electrode, and Ag/AgCl as a reference electrode.

Figure 1 shows the results of DPV measurements. In the absence of proteins, the E-aptasensor shows two distinctive peaks at 0.2 and −0.5 V (versus Ag/AgCl), which are due to the ferrocene label of Thrombin aptamer and the anthraquinone reporter of Lysozyme aptamer, respectively. Upon addition of thrombin (100 nmol) and lysozyme (100 nmol), about 40–50% decrease in these two DPV peaks were observed in 60 min (Figure 1a). When only lysozyme (100 nmol) was present in the assay solution, only the Lysozyme aptamer signaling peak was decreased (ca. 30%) along with little or no changes in the peak current due to Thrombin apatamer (Figure 1b). On the contrary, as shown in Figure 1c, only the ferrocene peak responded in a similar extent to the thrombin-containing solution (100 nmol). These results clearly demonstrated that the present multiprotein sensor has little or no cross effect in the protein detection and can selectively detect the target protein analytes.

In conclusion, owing to the simplicity of biosensor fabrication and no necessity of target labeling and washing, the present method has indeed some advantages in development of electronic aptasensors for multi-analytes on a single platform.

This research was supported by a grant from JSPS.

References and Notes

1 J. Liu, Z. Cao, Y. Lu, *Chem. Rev.* **2009**, *109*, 1948.
2 I. Willner, M. Zayat, *Angew. Chem., Int. Ed.* **2007**, *46*, 6408.
3 M. Famulok, J. S. Hartig, G. Mayer, *Chem. Rev.* **2007**, *107*, 3715.
4 Z. Tang, P. Mallikaratchy, R. Yang, Y. Kim, Z. Zhu, H. Wang, W. Tan, *J. Am. Chem. Soc.* **2008**, *130*, 11268.
5 E. E. Ferapontova, E. M. Olsen, K. V. Gothelf, *J. Am. Chem. Soc.* **2008**, *130*, 4256.
6 R. Kirby, E. J. Cho, B. Gehrke, T. Bayer, Y. S. Park, D. P. Neikirk, J. T. McDevitt, A. D. Ellington, *Anal. Chem.* **2004**, *76*, 4066.
7 C. Bock, M. Coleman, B. Collins, J. Davis, G. Foulds, L. Gold, C. Greef, J. Heil, J. S. Heilig, B. Hicke, M. N. Hurst, G. M. Husar, D. Miller, R. Ostroff, H. Petach, D. Schneider, B. Vant-Hull, S. Waugh, A. Weiss, S. K. Wilcox, D. Zichi, *Proteomics* **2004**, *4*, 609.
8 K. Stadtherr, H. Wolf, P. Lindner, *Anal. Chem.* **2005**, *77*, 3437.
9 J. R. Collett, E. J. Cho, J. F. Lee, M. Levy, A. J. Hood, C. Wan, A. D. Ellington, *Anal. Biochem.* **2005**, *338*, 113.
10 E. J. Cho, J. R. Collett, A. E. Szafranska, A. D. Ellington, *Anal. Chim. Acta* **2006**, *564*, 82.
11 J. A. Hansen, J. Wang, A.-N. Kawde, Y. Xiang, K. V. Gothelf, G. Collins, *J. Am. Chem. Soc.* **2006**, *128*, 2228.
12 F. Ricci, A. J. Bonham, A. C. Mason, N. O. Reich, K. W. Plaxco, *Anal. Chem.* **2009**, *81*, 1608.
13 J. Yoshizumi, S. Kumamoto, M. Nakamura, K. Yamana, *Analyst* **2008**, *133*, 323.
14 Z.-S. Wu, M.-M. Guo, S.-B. Zhang, C.-R. Chen, J.-H. Jiang, G.-L. Shen, R.-Q. Yu, *Anal. Chem.* **2007**, *79*, 2933.
15 Y. Lu, X. C. Li, L. M. Zhang, P. Yu, L. Su, L. Q. Mao, *Anal. Chem.* **2008**, *80*, 1883.
16 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.
17 With the present sensor fabrication, total amounts of redox-modified DNA duplexes on a gold electrode were 3–5 pmol cm^{-2}.
18 M. Watanabe, S. Kumamoto, M. Nakamaura, K. Yamana, *Bioorg. Med. Chem.* **2009**, *17*, 1494.

 © 2010 The Chemical Society of Japan