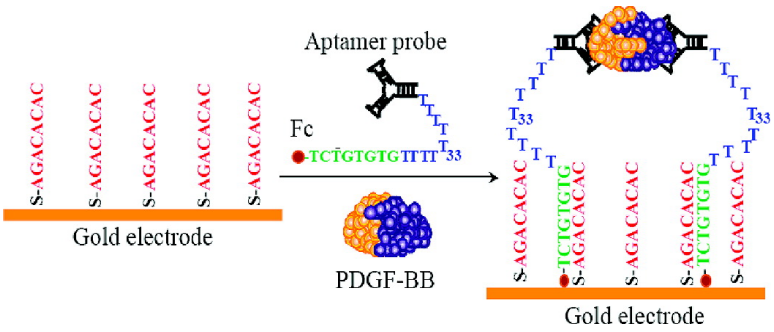
Communication

Electrochemical Aptasensor Based on Proximity-Dependent Surface Hybridization Assay for Single-Step, Reusable, Sensitive Protein Detection

Yan-Li Zhang, Yong Huang, Jian-Hui Jiang, Guo-Li Shen, and Ru-Qin Yu

J. Am. Chem. Soc., **2007**, 129 (50), 15448-15449 • DOI: 10.1021/ja0773047



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 2 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 11/22/2007

Electrochemical Aptasensor Based on Proximity-Dependent Surface Hybridization Assay for Single-Step, Reusable, Sensitive Protein Detection

Yan-Li Zhang, Yong Huang, Jian-Hui Jiang,* Guo-Li Shen, and Ru-Qin Yu*

State Key Laboratory for Chemo/biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, PRC

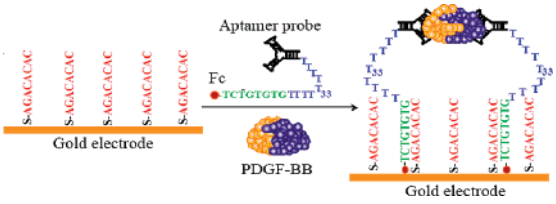
Received September 25, 2007; E-mail: jianhuijiang@hnu.cn; rqyu@hnu.cn

Aptamers are short single-stranded oligonucleotides selected for their high affinity in binding specifically to proteins or other targets.[1] While antibody-based immunoassay methodologies constitute routine tools for protein detection, the use of aptamer−protein recognition has emerged as a promising alternative due to enormous versatility of nucleic acid components in labeling, immobilization, signaling, and amplification. Aptamers have been established as ideal recognition elements for sensing technologies with mechanical,[2] optical,[3] or electronic readouts.[4]

Electrochemical detection is of particular significance in the development of aptasensors since it allows for high sensitivity and selectivity, simple instrumentation, as well as low endogenetic background. Many electrochemical strategies only incorporate the aptamer−target binding events into the sensor design, and the presence of target is signaled either via direct measurements of electrochemical parameters such as impedance[5] and potential[6] or through indirect detection of certain exogenous labels including enzymes[7] and nanoparticles.[8] Alternative designs exploit certain biochemical properties of aptamers as substrates of DNA enzymes[9] or conformational switches[10] besides the recognition affinity. In the context, electroactive labels have been a subject of intensive interest. Aptamer molecules frequently undergo extensive conformational changes on interacting with cognate ligands by adaptive binding events.[11] This constitutes a unique mechanism in the design of electrochemical aptasensors for thrombin,[12] cocaine,[13] and platelet-derived growth factor (PDGF),[14] where adaptive conformational switches modulate the distance of electroactive labels from the electrode, altering the redox current. Another conceptually distinct mechanism for electrochemical aptasensors is based on conformational changes induced by strand displacement or structure switching.[15,16] In this design, the adaptive binding of aptamer to target triggers the release of a short DNA fragment from its complementary aptamer sequence, which mediates conformational transition[15] and even dissociation[16] of the redox-modified DNA sequence at the electrode surface, thus furnishing a measurable electrochemical signal. Here we report the proof-of-principle of a novel electrochemical aptasensor for protein detection based on proximity-dependent surface hybridization. This aptasensor allows a single-step, signal-on, and reusable assay toward a model target, the homodimer of PDGF B-chain (PDGF-BB),[17] with high sensitivity and selectivity.

This proximity-dependent surface hybridization assay relies on simultaneous recognition of a target molecule by a pair of affinity probes followed by hybridization of the proximate affinity probes with surface-tethered oligonucleotide strands, as shown in Scheme 1. The aptasensor is fabricated by self-assembly of a short thiolated DNA oligonucleotide **1** on a ∼2 mm gold electrode via the alkanethiol moiety at the 5′-terminal (∼2.7×10^{13} strand/cm^2). A DNA aptamer **2** to PDGF-BB with a sequence extension at the 3′-end (Figure S1) is used as the affinity probe.[18] This aptamer constitutes the pair of affinity probes simultaneously recognizing PDGF-BB, a homodimer molecule. The aptamer probe has a 3′-ferrocene-labeled eight-nucleotide tail sequence that is complementary to the surface-tethered DNA strands with a predesigned low melting temperature (usually <20 °C). In the absence of target protein, the tail sequence does not associate with the surface-tethered strands because the complementary fragment is too short to promote effective annealing. When aptamer pairs simultaneously bind to PDGF-BB, the tail sequences are brought into close proximity with their local concentration increased substantially to allow the pair of tail sequences to hybridize together with the surface-tethered DNA strands.[3a,9a] Then the ferrocene labels of the tail sequence are drawn close to the electrode surface, producing a readily detectable redox current.

Scheme 1. Electrochemical Detection Strategy Based on Proximity-Dependent Surface Hybridization Assay[a]



[a] Simultaneous recognition of target brings two proximate aptamer probes, promoting the flanking Fc-labeled tail sequences to hybridize together with immobilized strands and triggering redox current. The absence of target only allows independent annealing of the tail sequences, disabling the hybridization of the short sequences due to predesigned low melting temperature.

The developed aptasensor demonstrated a signal-on architecture in response to the target. In the absence of PDGF-BB, only insignificant signal was observed in cyclic voltammograms (CV) (Figure 1A), evidencing that the hybridization of ferrocene-labeled aptamer probes with immobilized oligonucleotides was performed with extremely low efficiency. After reaction with 5 ng/mL of PDGF-BB, the CV curves showed a pair of well-defined current peaks at 0.152 and 0.256 V (vs SCE), a typical redox peak range of ferrocene. The differential pulse voltammograms (DPV) provided quite nice resolution of the response (Figure S2). One observed a small DPV peak (SD across four repetitive experiments ∼5%) around 0.208 V in the absence of target protein, which was presumed to arise from low-efficiency annealing of aptamer to immobilized strands. After reaction with 5 ng/mL of PDGF-BB, there appeared a strong DPV peak (SD across four repetitive experiments ∼4%) with a signal gain of ∼7-fold with reference to that for the blank. In contrast, other proteins (20 ng/mL) such as PDGF-AB, BSA, insulin, IgG, and thrombin did not produce significant alteration of DPV signals (Figure 1B), indicating that the fabricated aptasensor was not responsive to specific recognition of PDGF-AB by only an aptamer probe and nonspecific interaction between proteins and the aptamer probes. One thus reasoned that a simultaneous recognition of the target by two aptamer probes was a prerequisite for efficiently facilitating the hybridization of the aptamer probes with the surface-tethered strands. Moreover, one observed that there was no appreciable DPV signal (∼2 nA) in the control experiments with noncomplementary surface-tethered oligonucleotides **3**, suggesting that surface hybridization was essential in signaling the recognition events. Actually, simultaneous

10.1021/ja0773047 CCC: $37.00

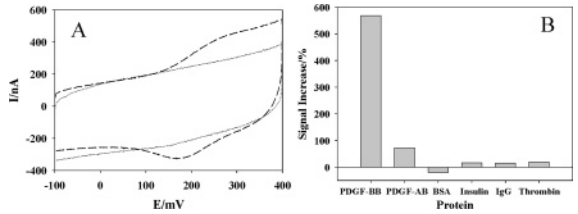


Figure 1. (A) CVs of aptasensor in 0.1 M $KClO_4$ before (solid line) and after (dashed line) reaction with 5 ng/mL of PDGF-BB. Potential scanning rate is 100 mV/s. (B) DPV peak currents for different proteins (PDGF-BB, 5 ng/mL; other proteins, 20 ng/mL). Reaction buffer is 10 mM PBS (pH 7.4, 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$).

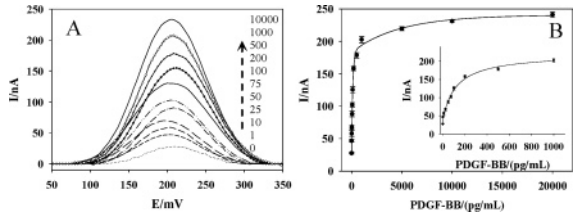


Figure 2. (A) Typical DPV response curves of aptasensor at varying PDGF concentration (pg/mL). (B) DPV peak currents for different PDGF concentrations. DPV is recorded in 0.1 M $KClO_4$. Error bars are standard deviation across four repetitive experiments. The inset gives responses in 0−1 ng/mL range.

recognition of target protein by two aptamer probes and hybridization of the aptamer probes with immobilized strands comprised two crucial steps in the proximity-dependent surface hybridization assay, which offered a possibility for highly selective detection of the target protein.

Figure 2 depicts typical DPV responses of the aptasensor to PDGF-BB of varying concentrations. One observed dynamically increased DPV current with increasing PDGF-BB concentration ranging from 1.0 pg/mL to 20 ng/mL. The saturated response gave a surface coverage of the ferrocene-labeled aptamers of $\sim 4.3 \times 10^{11}$ strand/cm^2. A high dose−response sensitivity was obtained in a three-decade concentration range from 1.0 pg/mL to 1.0 ng/mL with a readily achieved detection limit of 1.0 pg/mL. In additional to a substantially improved signal gain, this aptasensor gave a detection sensitivity $\sim 10^3$-fold better than that obtained with conformation switch based strategy.[14]

A close inspection of the CV currents versus different scan rates revealed that the peak current increased in linear correlation to the scan rate, featuring a typical surface-bound electrochemical process and confirming that ferrocene was confined to the electrode surface (Figure S3). A further impedance analysis of the interfacial processes (Figure S4) demonstrated that simultaneous hybridization of two aptamer probes **4** with a 3′-amino moiety that bound to PDGF-BB resulted in increased Faraday impedance, indicators of the surface binding events. Interestingly, hybridization of two aptamer probes **2** with a 3′-ferrocene label induced greatly decreased electrochemical impedance. This gave immediate evidence for surface confinement of the ferrocene labels that exhibited facilitated electron transfer kinetics.

Because the aptasensor only included a surface-tethered monolayer of oligonucleotides without preferred conformation, the sensor interface could be readily regenerated. It was found that the aptasensor could be reused over eight times without significant loss of sensitivity via washing with 1 M NaOH for 10 min at 50 °C. Presumably, the duplex structure might be collapsed in high alkaline solution due to the break of hydrogen bonds.

In conclusion, we developed a reusable electrochemical aptasensor for single-step detection of PDGF-BB based on proximity-dependent surface hybridization assay. The sensor required simultaneous recognition of the target by two aptamer probes to promote their efficient annealing with surface-immobilized oligonucleotides. The method could be applied immediately to detecting other proteins by using two aptamers with different binding sites. With a pair of antibody−oligonucleotide conjugates with different binding sites, it was possible to extend the strategy to immunoassay systems,[19] allowing the detection of a broad spectrum of proteins. Also, with different oligonucleotides, each specifically designed for individual protein, the strategy could be implemented for multiplex detection of multiple proteins in a densely packed sensing array format. In view of these advantages, the surface proximity-dependent surface hybridization assay, as a new electrochemical strategy, might create a universal methodology for developing high-performance biosensors in sensitive, selective, and reusable detection of proteins.

Acknowledgment. This work was supported by NSF (20675028) and Ministry of Education (NCET-04-0768) of China.

Supporting Information Available: Detailed description of the experimental procedures and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Tuerk, C.; Gold, L. *Science* **1990**, *249*, 505−510. (b) Ellington, A. D.; Szostak, J. W. *Nature* **1990**, *346*, 818−822.

(2) (a) Savran, C. A.; Knudsen, S. M.; Ellington, A, D.; Manalis, S. R. *Anal. Chem.* **2004**, *76*, 3194−3198. (b) Bine, A.; Minunni, M.; Tombelli, S.; Centi, S.; Mascini, M. *Anal. Chem.* **2007**, *79*, 3016−3019.

(3) (a) Fredriksson, S.; Gullberg, M.; Jarvius, J.; Olsson, C.; Pietras, K.; Margrét, S. G.; Östmank, A.; Landegren, U. *Nat. Biotechnol.* **2002**, *20*, 473−477. (b) Nutiu, R.; Li, Y. *J. Am. Chem. Soc.* **2003**, *125*, 4771−4778. (c) Yang, L.; Fang, C. W.; Cho, E. J.; Ellington, A. D. *Anal. Chem.* **2007**, *79*, 3320−3329.

(4) Willner, I.; Zayats, M. *Angew. Chem., Int. Ed.* **2007**, *46*, 6408−6418.

(5) (a) de los Santos-Alvarez, N.; Lobo-Castanon, M. J.; Mirando-Ordieres, A. J.; Tunon-Blanco, P. *J. Am. Chem. Soc.* **2007**, *129*, 3808−3809. (b) Radi, A. E.; Acero Sanchez, J. L.; Baldrich, E.; O'Sullivan, C. K. *Anal. Chem.* **2005**, *77*, 6320−6323.

(6) (a) So, H. M.; Won, K.; Kim, Y. H.; Kim, B. K.; Ryu, B. H.; Na, P. S.; Kim, H.; Lee, J. O. *J. Am. Chem. Soc.* **2005**, *127*, 11906−11907. (b) Maehashi. K.; Katsura, T.; Kerman, K.; Takamura, Y.; Matsumoto, K.; Tamiya, E. *Anal. Chem.* **2007**, *79*, 782−787.

(7) Centi, S.; Tombelli, S.; Minunni, M.; Mascini, M. *Anal. Chem.* **2007**, *79*, 1466−1473.

(8) (a) Hansen, J. A.; Wang, J.; Kawde, A. N.; Xiang, Y.; Gothelf, K. V.; Collins, G. *J. Am. Chem. Soc.* **2006**, *128*, 2228−2229. (b) Polsky, R.; Gill, R.; Kaganovsky, L.; Willner, I. *Anal. Chem.* **2006**, *78*, 2268−2271.

(9) (a) Giusto, D. A. D.; Wlassoff, W. A.; Gooding, J. J.; Messerle, B. A.; King, G. C. *Nucleic Acids Res.* **2005**, *33*, e64. (b) Le Floch, F.; Ho, H. A.; Leclerc, M. *Anal. Chem.* **2006**, *78*, 4727−4731. (c) Zhou, L.; Ou, L. J.; Chu, X.; Shen, G. L.; Yu, R. Q. *Anal. Chem.* **2007**, *79*, 7492−7500.

(10) (a) Zayats, M.; Huang, Y.; Gill, R.; Ma, C.-A.; Willner, I. *J. Am. Chem. Soc.* **2006**, *128*, 13666−13667. (b) Bang, G. S.; Cho, S.; Kim, B. G. *Biosens. Bioelectron.* **2005**, *21*, 863−870.

(11) Piganeau, N.; Jenne, A.; Thuillier, V.; Famulok, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 4369−4373.

(12) (a) Xiao, Y.; Lubin, A. L.; Heeger, A. J.; Plaxo, K. W. *Angew. Chem., Int. Ed.* **2005**, *44*, 5456−5459. (b) Radi, A. E.; Acero Sánchez, J. L.; Baldrich, E.; O'Sullivan, C. K. *J. Am. Chem. Soc.* **2006**, *128*, 117−124.

(13) Baker, B. R.; Lai, R. Y.; Wood, M. S.; Doctor, E. H.; Heeger, A. J.; Plaxo, K. W. *J. Am. Chem. Soc.* **2006**, *128*, 3138−3139.

(14) Lai, R. Y.; Plaxo, K. W.; Heeger, A. J. *Anal. Chem.* **2007**, *79*, 229−233.

(15) (a) Xiao, Y.; Piorek, B. D.; Plaxco, K. W.; Heeger, A. J. *J. Am. Chem. Soc.* **2005**, *127*, 17990−17991. (b) Zuo, X.; Song, S.; Zhang, J.; Pan, D.; Wang, L.; Fan, C. *J. Am. Chem. Soc.* **2007**, *129*, 1042−1043.

(16) Wu, Z. S.; Guo, M. M.; Zhang, S. B.; Chen, C. R.; Jiang, J. H.; Shen, G. L.; Yu, R. Q. *Anal. Chem.* **2007**, *79*, 2933−2939.

(17) Heldin, C. H.; Westermark, B. *Physiol. Rev.* **1999**, *79*, 1283−1316.

(18) To ensure accessibility of two proximate aptamers to immobilized oligonucleotides, a long flexible spacer sequence (41 dT) was designed in the aptamer probe. The spacer length, either estimated by molecular modeling or optimized using experiments, could be varied over a considerable range with negligible effect on the signal gain. Self-assembly of thiolated capturing probes was recommended due to its high surface coverage and small distance between the immobilized probes.

(19) Gullberg, M.; Gústafsdóttir, S. M.; Schallmeiner, E.; Jarvius. J.; Bjamegård, M.; Betsholtz, C.; Landegren, U.; Fredriksson, S. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 8420−8424.

JA0773047