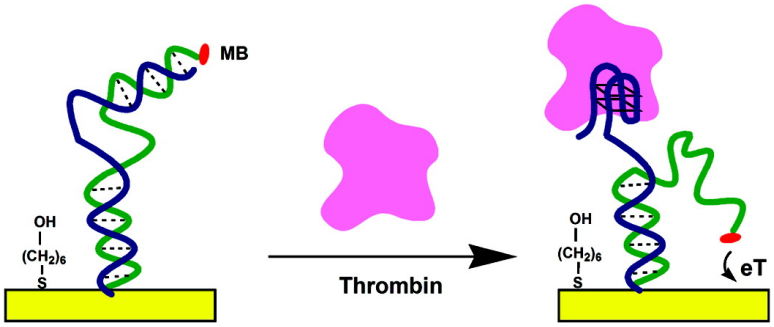
Communication

A Reagentless Signal-On Architecture for Electronic, Aptamer-Based Sensors via Target-Induced Strand Displacement

Yi Xiao, Brian D. Piorek, Kevin W. Plaxco, and Alan J. Heeger

J. Am. Chem. Soc., **2005**, 127 (51), 17990-17991• DOI: 10.1021/ja056555h • Publication Date (Web): 01 December 2005



More About This Article

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

- Supporting Information
- Links to the 51 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 12/01/2005

A Reagentless Signal-On Architecture for Electronic, Aptamer-Based Sensors via Target-Induced Strand Displacement

Yi Xiao,[†] Brian D. Piorek,[‡] Kevin W. Plaxco,*[,‡,§] and Alan J. Heeger[†]

Department of Physics, Materials Department, and Institute for Polymers and Organic Solids, Program in BioMolecular Science and Engineering, and Department of Chemistry and Biochemistry, University of California, Santa Barbara, California 93106

Received September 30, 2005; E-mail: kwp@chem.ucsb.edu

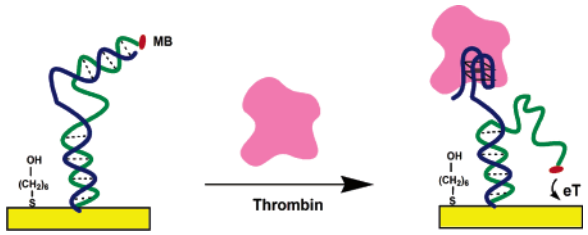
Aptamers, short oligonucleotides selected in vitro for specific, high-affinity binding to a broad range of molecular targets,[1−4] are considered promising recognition elements for biosensor applications,[5−16] as illustrated by a recent report of 10 pM thrombin detection using an aptamer-directed rolling-circle amplification assay.[17] We have previously described[16] a new class of electronic, aptamer-based (E-AB) sensors that are sensitive and selective enough to work directly in blood and other complex, contaminant-ridden materials and, in contrast to other promising approaches,[10−15,17] do not require processing steps or the addition of any exogenous reagents. E-AB signal generation occurs when a binding-induced conformational change significantly alters electron tunneling from a redox-tagged, electrode-bound aptamer to the sensing electrode.

While our previously described E-AB sensor is characterized by the desirable features of being reagentless, reusable, and selective enough to employ directly in blood,[16] it suffers from the limitations associated with being a "signal-off" architecture, in which the presence of target reduces rather than increases signal strength. As such, the architecture suffers from the problem of limited signal gain since, at most, only 100% of the signal can be suppressed. Moreover, for the signal-off sensor, contaminants that degrade the aptamer or its redox label can give rise to "false positives" that are difficult to distinguish from signals arising from the binding of authentic analyte. These problems would be alleviated by a "signal-on" mechanism that, instead, produces significant increases in peak current upon target recognition.

Here we describe a presumably general signal-on architecture for E-AB sensors that is a simple modification of our original sensor design and leads to order of magnitude increases in signal gain and sensitivity. To modify the system, we have introduced a short, methylene blue (MB)-tagged oligonucleotide that hybridizes with both the thrombin-binding portion of our originally employed aptamer[16] and the DNA sequence linking the aptamer to the electrode. The formation of these regions of rigid, duplex DNA prevents the MB tag from approaching the electrode surface (Scheme 1), suppressing Faradaic currents. Thrombin binding stabilizes the alternative G-quadruplex conformation of the aptamer, liberating the 5′ end of the tagged oligonucleotide to produce a flexible, single-stranded element. This, in turn, allows the MB tag to collide with the electrode surface, producing a readily detectable Faradaic current.

The signal-on E-AB sensor is constructed by covalently attaching a thiolated DNA aptamer (**1**) to a gold electrode via well-established self-assembled monolayer chemistry.[18] The DNA (**1**) is predesigned by introducing into the 15-base sequence from the 3′ end that specifically binds to thrombin with high affinity. A MB-tagged,

Scheme 1. The Proposed Mechanism of the Signal-On Electronic, Aptamer-Based (E-AB) Sensor



partially complementary DNA (**2**) is added and forms a double-stranded complex with the electrode-bound aptamer. We presume that the immobilized aptamer (**1**) is in a conformational equilibrium between duplex DNA conformer and the binding-competent G-quadruplex conformer.[11] Because thrombin only binds the folded aptamer, protein binding drives the equilibrium from duplex to the G-quadruplex, liberating the 5′ end of the MB-tagged oligonucleotide as a flexible, single-stranded element and thus producing a detectable electronic signal.

To fabricate the sensor, a clean gold surface was reacted with a solution of thiolated thrombin binding aptamer (**1**), 0.8 μM including 8 μM TCEP (tris-(2-carboxyethyl)phosphine hydrochloride, which is included to reduce disulfide bonded oligos) in 200 mM Tris-HCl buffer, pH 7.4, for 16 h at room temperature. The resulting surface was washed with the Tris-HCl buffer, and then the (**1**)-functionalized gold surface was treated with 1 mM 1-mercapto-hexanol in 10 mM Tris-HCl buffer, pH 7.4, for 1 h. The resulting monolayer-functionalized surface was treated with the MB-tagged complementary DNA (**2**), 1.0 μM, in Perfect Hyb plus hybrization buffer (Sigma) (1X), for 3 h to yield the ds-DNA-aptamer assembly on the surface. Electrochemical measurements indicate that the DNA (**2**) is loaded on the gold surface at 3.0 ± 0.2 pmol·cm^{-2}.

The signal-on E-AB sensor is responsive to its target protein. In the absence of thrombin, we observe only small, highly reproducible (standard deviation across four electrodes ∼4%) Faradaic currents (Figure 1A, 0 nM thrombin). We presume this residual peak arises because the surface-immobilized aptamer (**1**) is in a conformational equilibrium between a duplex DNA and the binding-competent G-quadruplex.[11] As we add thrombin in solution, the surface loading of bound thrombin increases, boosting the Faradaic current until a 3-fold signal gain is obtained at a target concentration of ∼260 nM (Figure 1A).

In contrast to the signal-off E-AB sensor, for which thrombin concentrations of as high as 800 nM produce only a 40% signal decrease, the signal-on E-AB sensor produces an approximately 270% signal gain at only 256 nM thrombin. This improved signal gain leads to improved signal robustness and sensitivity. For example, whereas 19 nM thrombin produces only a 7% signal drop in our original sensor, this same target concentration produces a

[†] Department Physics, Materials Department, and Institute for Polymers and Organic Solids.
[‡] Program in BioMolecular Science and Engineering.
[§] Department of Chemistry and Biochemistry.

10.1021/ja056555h CCC: $30.25

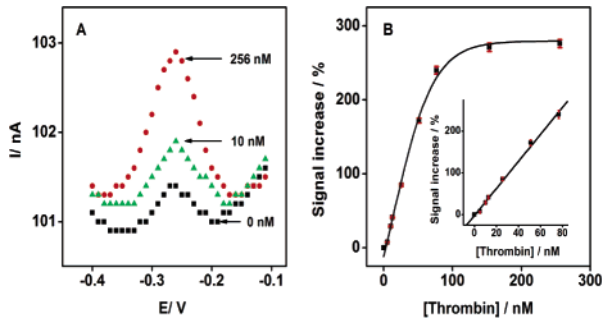


Figure 1. (A) Alternating current voltammetric (ACV) curves of the ds-DNA-aptamer functionalized surfaces obtained in 20 mM Tris-HCl, pH 7.4 with 140 mM NaCl, 20 mM MgCl, and 20 mM KCl at various thrombin concentrations. (B) A dose−response curve for the signal-on E-AB thrombin sensor. The illustrated error bars represent the standard deviation of four measurements conducted with a single electrode at each thrombin concentration. Multiple electrodes were used to collect the entire data set. (Relative sensor response, in percent change, is employed because this is more reproducible electrode-to-electrode than absolute current change.)

60% signal increase in the new architecture. As a result, the signal-on sensor allows us to detect thrombin at concentrations as low as 3 nM. The dynamic range of the E-AB sensor (Figure 1B) covers physiologically relevant concentrations, which range from a few nanomolar in resting blood to several hundred nanomolar when the clotting cascade is activated.[19]

Control experiments reveal that, when coupled with its complementary MB-tagged DNA (**4**), a thiolated oligonucleotide (**3**) of identical sequence composition and 81% sequence identity with the DNA thrombin aptamer (**1**) but known not to bind to thrombin[2] does not exhibit any measurable signal change when challenged with thrombin (see Supporting Information, S3). Similarly, an E-AB sensor built with the thrombin-binding aptamer does not exhibit any measurable response when challenged with 300 nM bovine serum albumin (see Supporting Information, S4). These results imply that release of the MB tag and the subsequent increase in electron transfer require the formation of a specific aptamer−thrombin complex.

The signal-on E-AB sensor we have described here requires that the 3′ end of the MB-tagged oligonucleotide (**2**) remains physically associated with the electrode after target binding (Scheme 1, right). To ensure this, we have designed the system to form a 12-base duplex DNA with the DNA sequence that covalently links the aptamer to the electrode surface. In the optimized hybridization buffer we have employed, this duplex element is stable for more than 24 h. Nevertheless, fully covalent systems should significantly improve the stability and versatility of this sensor platform. Similarly, the modified signal-on system couples binding to an unfavorable reaction (breaking the duplex) and thus no doubt reduces the affinity of the aptamer for its target. Despite this, the 10-fold improvement in signal gain produced by the new, signal-on architecture allows us to detect much lower concentrations of thrombin than was possible with our first generation sensor. Further optimization of this set of competing equilibria should lead to further reductions in the sensor's detection limit. However, unlike our original signal-off E-AB sensor architecture, the sensor described here is not reusable; the hybridization that retains the signaling oligonucleotide (**2**) in proximity with the electrode will not withstand the solvent conditions required to disrupt thrombin binding.[16]

The signal-on E-AB sensor architecture we have described is applicable to a wide range of aptamers. We base this speculation on the observation that Ellington[11a] has rationally engineered a normally well-folded aptamer into an optical aptamer beacon by adding a short sequence to the 5′ end which is complementary to the 3′ end of the aptamer. In the absence of target, the modified aptamer preferentially adopts a stem-loop structure inhibiting fluorescence from a dye/quencher pair conjugated to the two termini. In the presence of target, a ligand-binding G-quadruplex is favored, enhancing fluorescence. Given that virtually any aptamer can be so modified, and given that aptamers can generally be immobilized without significant loss of activity,[12a] it appears that the E-AB sensor architecture provides a straightforward means of converting any well-folded aptamer into a sensitive, selective, reagentless, electronic sensor. This approach, therefore, offers a new direction for the rapid, convenient detection of protein and small molecule analytes.

Acknowledgment. This work was supported in part by NIH EB002046, by NSF DMR 0099843, and by the Institute for Collaborative Biotechnologies through Grant DAAD19-03-D-0004 from the U.S. Army Research Office. We thank Brian Baker and Kevin Cash for providing helpful commentary.

Supporting Information Available: The experimental conditions for preparation of thrombin aptamer modified electrode and control experiments are provided. This material is available free of charge via Internet at http://pubs.acs.org.

References

(1) Luzi, E.; Minunni, M.; Tombeli, S.; Mascini, M. *Trends Anal. Chem.* **2003**, *11*, 810−818.
(2) Ellington, A. D.; Szostak, J. W. *Nature* **1990**, *346*, 818−822.
(3) (a) Tuerk, C.; Gold, L. *Science* **1990**, *249*, 505−510. (b) Robertson, M. P.; Joyce, G. F. *Nature* **1990**, *344*, 467−470.
(4) Bock, L. C.; Griffin, L. C.; Latham, J. A.; Vermass, E. H.; Toole, J. J. *Nature* **1992**, *355*, 564−566.
(5) Kleinjung, F.; Klussman, S.; Erdmann, V. A.; Scheller, F. W.; Furste, J. P.; Bier, F. F. *Anal. Chem.* **1998**, *70*, 328−331.
(6) Potyrailo, R. A.; Conrad, R. C.; Ellington, A. D.; Hieftje, G. M. *Anal. Chem.* **1998**, *70*, 3419−3425.
(7) Stojanovic, M. N.; de Prada, P.; Landry, D. W. *J. Am. Chem. Soc.* **2001**, *123*, 4928−4931.
(8) Yamamoto, R.; Baba, T.; Kumar, P. K. *Genes Cells* **2000**, *5*, 389−396.
(9) Savran, C. A.; Knudsen, S. M.; Ellington, A. D.; Manalis, S. R. *Anal. Chem.* **2004**, *76*, 3194−3198.
(10) Fang, X. H.; Sen, A.; Vicens, M.; Tan, W. H. *ChemBioChem* **2003**, *4*, 829−834.
(11) (a) Hamaguchi, N.; Ellington, A. D.; Stanton, M. *Anal. Biochem.* **2001**, *294*, 126−131. (b) Dittmer, W. U.; Reuter, A.; Simmel, F. C. *Angew. Chem., Int. Ed.* **2004**, *43*, 3550−3553.
(12) (a) Bock, L. C.; Griffin, L. C.; Latham, J. A.; Vermaas, E. H.; Toole, J. J. *Nature* **1992**, *355*, 564−566. (b) Macaya, R. F.; Waldron, J. A.; Beutel, B. A.; Gao, H. T.; Joesten, M. E.; Yang, M. H.; Patel, R.; Bertelsen, A. H.; Cook, A. F. *Biochemistry* **1995**, *34*, 4478−4492. (c) Padmanabhan, K.; Tulinsky, A. *Acta Crystallogr.* **1996**, *D52*, 272−282.
(13) Ho, H. A.; Leclerc, M. *J. Am. Chem. Soc.* **2004**, *126*, 1384−1387.
(14) Pavlov, V.; Xiao, Y.; Shlyahovsky, B.; Willner, I. *J. Am. Chem. Soc.* **2004**, *126*, 11768−11769.
(15) Fukusho, S.; Furusawa, H.; Okahata, Y. *Chem. Commun.* **2002**, 88−89.
(16) Xiao, Y.; Lubin, A. A.; Heeger, A. J.; Plaxco, K. W. *Angew. Chem., Int. Ed.* **2005**, *44*, 5456−5459.
(17) Di Giusto, D. A.; Wlassoff, W. A.; Gooding, J. J.; Messerle, B. A.; King, G. C. *Nucleic Acids Res.* **2005**, *33*, e64.
(18) Fan, C. H.; Plaxco, K. W.; Heeger, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 9134−9137.
(19) Lee, M.; Walt, D. *Anal. Biochem.* **2000**, *282*, 142−146.

JA056555H