J|A|C|S
COMMUNICATIONS

Published on Web 01/18/2007

A Target-Responsive Electrochemical Aptamer Switch (TREAS) for Reagentless Detection of Nanomolar ATP

Xiaolei Zuo,[†,‡] Shiping Song,[†] Jiong Zhang,[†,‡] Dun Pan,[†] Lihua Wang,[†] and Chunhai Fan*[,†]

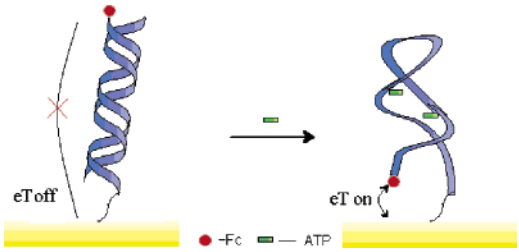
Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China, and Graduate School of the Chinese Academy of Sciences, Beijing 100039, China

Received September 30, 2006; E-mail: fchh@sinap.ac.cn

Aptamers are artificial oligonucleotide receptors originated from in vitro selection (SELEX).[1] In principle, aptamers with high specificity and affinity can be selected for any given target, ranging from small molecules to large proteins and even cells.[2] Therefore, aptamers are widely recognized as highly promising tools for a variety of important applications.[3,4] Aptamers are particularly useful as the biosensing element as they are chemically stable, readily available, and offer high flexibility in biosensor design.[5−11] Recently, Heeger, Plaxco, and others developed a series of novel electrochemical aptamer-based (E-AB) sensors for thrombin, cocaine, and potassium,[12−14] an analogous version to the electrochemical DNA (E-DNA) sensor.[8,15] These E-AB sensors are based on binding-induced conformational changes of redox-tagged and surface-confined aptamers, which have proven highly sensitive and selective.[12−14] Also, because E-AB sensors are electrochemistry-based, they are inherently fast, portable, and cost-effective. However, since E-AB relies on unique structures of aptamers, these sensors have to be designed case-by-case for different aptamer−target pairs. As a step further, Xiao et al. recently developed a potentially generalizable E-AB sensor for thrombin by using target-induced strand displacement.[16] Here we report a target-responsive electrochemical aptamer switch (TREAS), which is a signal-on sensor featuring both generalizability and simplicity in design, toward reagentless detection of adenosine triphosphate (ATP) with high sensitivity and selectivity.

We employed an in vitro selected 27-base anti-ATP aptamer, which possesses high affinity for ATP while not for its analogues, cytidine triphosphate (CTP), guanosine triphosphate (GTP), and uridine triphosphate (UTP).[17] The anti-ATP aptamer dually labeled with 3′-SH and 5′-ferrocene is self-assembled on gold electrodes in its duplex form (Scheme 1). We reason that ferrocene is distal to the electrode surface, thus cannot efficiently exchange electrons with the underlying electrodes due to large distance separation (∼10 nm) in this eT OFF state. In the presence of the target ATP, the tertiary aptamer structure is stabilized, which responsively denatures the duplex and liberates the complementary DNA, similar to the aptamer structural switch in solution.[18] As a consequence of this structural switch from the duplex to the tertiary aptamer structure (duplex-to-aptamer), the ferrocene moiety approaches the electrode surface and generates measurable electrochemical signals (eT ON). Of note, compared to the E-AB thrombin sensor reported by Xiao et al.,[16] our TREAS is similarly generalizable while it has several advantages. First, the sensor architecture is simpler. The sensing DNA strand of E-AB contains three parts, aptamer region (for recognition), duplex region (structural support), and spacer region (linkage), thus is inherently longer than the corresponding TREAS sensing strand (only aptamer sequence). Second, TREAS has two well-defined "ON" and "OFF" states due to its rigid structures in both cases. In contrast, the ON state of E-AB is in a floppy structure, thus electrochemical signals come from dynamic collision between the electrode and the redox moiety, which may attenuate the signal gain.

Scheme 1. The TREAS Strategy for ATP Detection[a]



[a] In the initial state, the ATP aptamer forms a duplex and ferrocene is distal to the electrode surface (et OFF; et stands for electron transfer); after reaction with ATP, the aptamer forms a tertiary structure with ATP and liberates its complementary strand. Ferrocene is proximal to the electrode surface (et ON) in this state.

We first employed a model system to test this proposed TREAS strategy, which involves two different duplexes self-assembled on gold electrodes. Both duplexes contain one thiolated strand at either the 5′- or 3′-terminus, while the complementary strands are tagged with ferrocene at the 5′-terminus. Of note, the 5′-thiolated one is analogous to the eT OFF state of TREAS (duplex) and the 3′-thiolated one to the eT ON state (aptamer). Indeed, we found a pair of well-defined CV peaks corresponding to the reduction and oxidation of ferrocene for the proximal ferrocene (Supporting Information, Figure 1-S). In contrast, we only found a very small signal in cyclic voltammetry (CV) for the distal ferrocene (the small signal might be attributed to incomplete hybridization and resulting residual ssDNA at the surface). This clearly demonstrates that the redox reaction of ferrocene is highly distance-dependent, as predicted by the Marcus electron transfer theory. It is worthwhile to point out that this phenomenon is different from the redox reaction of methylene blue, which is insensitive to its position on DNA duplexes. This is because methylene blue, while not ferrocene, is a DNA intercalator, and its electron communication with electrodes is coupled with highly efficient electron transfer within π-stacked base pairs.[19]

We then evaluated TREAS both in the absence and in the presence of ATP by using CV and square-wave voltammetry (SWV). Similar to that in the model system, there was only a small signal for TREAS in the duplex state (eT OFF). After challenging TREAS with 1 mM ATP, we observed a pair of well-defined CV peaks corresponding to the redox reaction of ferrocene (eT ON, Figure 2-S in the Supporting Information). SWV offers better resolution than CV, and we observed the similar trend in SWV. There was an intense SWV peak after reaction with 1 mM ATP, with a signal gain of ∼10-fold compared to that in the absence of ATP. The SWV peak current gradually increased along with the

[†] Shanghai Institute of Applied Physics, Chinese Academy of Sciences.
[‡] Graduate School of the Chinese Academy of Sciences.

10.1021/ja067024b CCC: $37.00

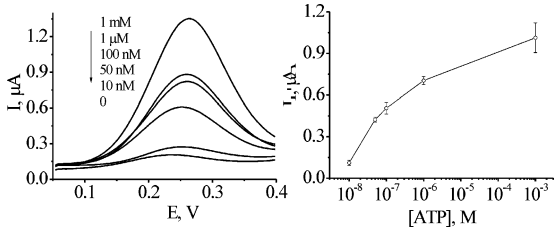


Figure 1. Representative square-wave voltammograms for the duplex modified electrode after reaction with various concentrations of ATP, ranging from 10 nM to 1 mM, in 10 mM HEPES containing 50 mM $NaClO_4$ (left panel). Plot for ATP concentration versus SWV peak current for TREAS. Data were averaged from at least three independent experiments (right panel).

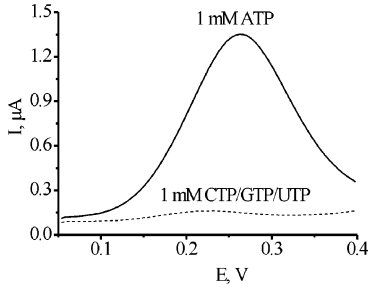


Figure 2. Square-wave voltammograms for the duplex modified electrode after reaction with 1 mM ATP (solid line) and a mixture of 1 mM CTP, 1 mM GTP, and 1 mM UTP (dashed line) in 10 mM HEPES containing 50 mM $NaClO_4$.

increase of ATP concentration ranging from 10 nM to 1 mM (Figure 1). This detection sensitivity of TREAS compares favorably with other reported aptamer-based ATP sensors (usually nM−mM).[6,11,20] However, further optimization is still necessary in order to match the sensitivity of commercially available luciferase-based ATP assays (∼0.1 nM).

We also electrochemically interrogated the ferrocene redox reaction. In the eT ON state, we found that CV peak currents of ferrocene were linearly proportional to scan rates, implying that the ferrocene electrochemistry was a surface-confined process (Supporting Information, Figure 3-S). The apparent heterogeneous electron transfer rate of ferrocene was determined to be 38 s^{-1}.[21] This rate is apparently low given that ferrocene is only approximately 1 nm away from the electrode in the eT ON state. This possibly suggests that ferrocene is in a flexible conformation due to the "soft" C6 linker, which increases thermodynamic and kinetic dispersion of ferrocene redox and lowers the apparent heterogeneous electron transfer rate.

TREAS is a selective sensor for ATP molecules. We challenged TREAS with 1 mM ATP and a mixture of ATP analogues, CTP, GTP, and UTP (all of 1 mM concentration). Importantly, the SWV signal for the analogue mixture was only as small as the background (in the absence of ATP), approximately 10-fold lower than that for 1 mM ATP (Figure 2). This excellent selectivity arises not only from the high specificity of anti-ATP aptamer but also from the additional stringency due to the competition between the duplex and the aptamer structure. In order to perform ATP assays in real samples, we employed TREAS to directly detect cellular ATP. We obtained a prominent SWV signal for freshly lysed cells (ATP present). In contrast, only a small residual signal was observed after storing cell lysates for 24 h (ATP absent) (Figure 4-S, Supporting Information). This clearly shows that TREAS might become a promising probe for cellular ATP assays.

In summary, we have described a novel TREAS strategy for ATP assays, which has several combined advantages. First, as with other E-DNA and E-AB sensors,[12,15,16] TREAS is inherently resistant to nonspecific interferences and highly usable in complex real samples. Second, TREAS is a reagentless sensor since both the recognition element (aptamer) and the signaling element (ferrocene) have been integrated in a surface-confined configuration. Third, TREAS is a signal-on sensor, which excels previously described signal-off sensors in both selectivity and multiplexibility.[12,15] Fourth, given the simplicity in design of TREAS, it is fairly easy to generalize this strategy to detect a spectrum of targets. In view of these advantages, we expect that this TREAS, as a novel strategy in the E-AB family, may offer a new direction in design of high-performance electrochemical biosensors for sensitive, selective, and reagentless detection of a wide spectrum of analytes.

Acknowledgment. We thank the financial support from National Natural Science Foundation (60537030 and 20404016), Shanghai Municipal Commission for Science and Technology (0652nm006), Ministry of Science and Technology (2006CB933000), Shanghai Rising-Star Program, and Chinese Academy of Sciences.

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) Ellington, A. D.; Szostak, J. W. *Nature* **1990**, *346*, 818−822.
(2) Hermann, T.; Patel, D. J. *Science* **2000**, *287*, 820−825.
(3) Lin, C.; Katilius, E.; Liu, Y.; Zhang, J.; Yan, H. *Angew. Chem., Int. Ed.* **2006**, *45*, 5296−5301.
(4) Lee, J. F.; Stovall, G. M.; Ellington, A. D. *Curr. Opin. Chem. Biol.* **2006**, *10*, 282−289.
(5) He, F.; Tang, Y.; Wang, S.; Li, Y.; Zhu, D. *J. Am. Chem. Soc.* **2005**, *127*, 12343−12346.
(6) Liu, J.; Lu, Y. *Angew. Chem., Int. Ed.* **2005**, *45*, 90−94.
(7) Ueyama, H.; Takagi, M.; Takenaka, S. *J. Am. Chem. Soc.* **2002**, *124*, 14286−14287.
(8) Fan, C.; Plaxco, K. W.; Heeger, A. J. *Trends Biotechnol.* **2005**, *23*, 186−192.
(9) Ohtani, Y.; Yamana, K.; Nakano, H. *Nucleic Acids Res. Suppl.* **2002**, 169−170.
(10) Jhaveri, S. D.; Kirby, R.; Conrad, R.; Maglott, E. J.; Bowser, M.; Kennedy, R. T.; Glick, G.; Ellington, A. D. *J. Am. Chem. Soc.* **2000**, *122*, 2469−2473.
(11) Zayats, M.; Huang, Y.; Gill, R.; Ma, C. A.; Willner, I. *J. Am. Chem. Soc.* **2006**, *128*, 13666−13667.
(12) Xiao, Y.; Lubin, A. A.; Heeger, A. J.; Plaxco, K. W. *Angew. Chem., Int. Ed.* **2005**, *44*, 5456−5459.
(13) Baker, B. R.; Lai, R. Y.; Wood, M. S.; Doctor, E. H.; Heeger, A. J.; Plaxco, K. W. *J. Am. Chem. Soc.* **2006**, *128*, 3138−3139.
(14) Radi, A.-E.; Sanchez, J. L. A.; Baldrich, E.; O'Sullivan, C. K. *J. Am. Chem. Soc.* **2006**, *128*, 117−124.
(15) Fan, C.; Plaxco, K. W.; Heeger, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 9134−9137.
(16) Xiao, Y.; Piorek, B. D.; Plaxco, K. W.; Heeger, A. J. *J. Am. Chem. Soc.* **2005**, *127*, 17990−17991.
(17) Huizenga, D. E.; Szostak, J. W. *Biochemistry* **1995**, *34*, 656−665.
(18) Nutiu, R.; Li, Y. *J. Am. Chem. Soc.* **2003**, *125*, 4771−4778.
(19) Boon, E. M.; Ceres, D. M.; Drummond, T. G.; Hill, M. G.; Barton, J. K. *Nat. Biotechnol.* **2000**, *18*, 1096−1100.
(20) Wang, J.; Jiang, Y.; Zhou, C.; Fang, X. *Anal. Chem.* **2005**, *77*, 3542−3546.
(21) Tender, L.; Carter, M. T.; Murray, R. W. *Anal. Chem.* **1994**, *66*, 3173−3181.

JA067024B