Target-induced strand release (TISR) from aptamer–DNA duplex: A general strategy for electronic detection of biomolecules ranging from a small molecule to a large protein†

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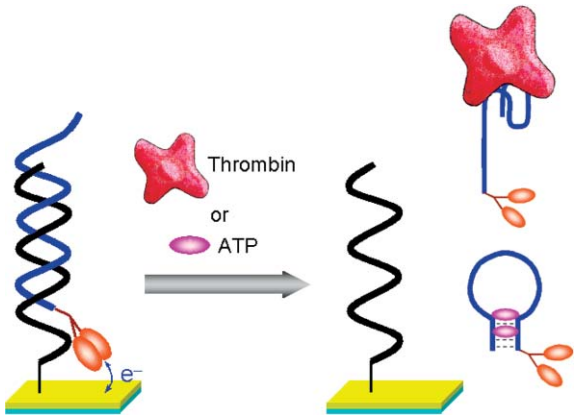
Received 10th December 2007, Accepted 17th December 2007
First published as an Advance Article on the web 10th January 2008
DOI: 10.1039/b719089c

We describe a general strategy for electronic aptamer-based biosensor that is based on target-induced strand release (TISR) of a redox-modified aptamer from the aptamer-capture DNA duplex bound on an electrode.

Aptamers, originating from *in vitro* selection,[1,2] are short oligonucleotides that act as receptors for a wide range of targets including small molecules, proteins, and cells.[3] With their high affinity and selectivity for target molecules, aptamers have been widely used in biosensor applications.[3–14] Among these applications, electrochemical methods have been utilized as transduction elements in the sensors[10–14] because they can offer label-free, rapid, and sensitive assays. Because electronic aptamer-based (E-AB) sensors provide useful tools in many biological applications such as drug screening, protein profiling, and medicinal diagnostics, development of such sensors is a current subject of intense research.

Most E-AB biosensors so far reported[10–14] are based on target-induced conformational changes or structural switching of redox-tagged aptamers bound to electrodes. The presence of target ligands can be determined by changes in the electronic signal due to the different electron transfer efficiency from the signal in the absence of target. However, this difference is generally not large and is dependent upon the aptamers used, in the case where single strand aptamers were installed into E-AB sensors.[10,11] To avoid this difficulty, aptamer–DNA duplexes have been used in biosensor architecture.[9,12–14] Relatively large changes in the aptamer conformation can be induced, giving improved signals in the detection of biomolecules.[12–14] In these situations, there is high demand to develop E-AB sensors with a different detection scheme that is applicable to a wide range of target biomolecules.

We describe here a simple yet convenient approach to E-AB biosensors. Our approach does not rely solely on conformational changes in aptamers, but rather is based on target-induced strand release (TISR) of a redox-modified aptamer from the aptamer-capture DNA duplex bound on an electrode. The concept of our strategy is depicted in Scheme 1. In the initial aptamer–DNA duplex, the redox-tag is confined near the electrode surface, giving an intense electrochemical signal. The binding of the target to the aptamer should lead to release of the aptamer strand through an aptamer structural switch.[7,9,12–14] Subsequent diffusion of the target-bound aptamer into the solution should result in significant reduction of the redox signal. The design and fabrication of our E-AB biosensors are simple and easy. Aptamers are singly modified at the 5′-terminus with an anthraquinone-redox-tag. The capture DNA is attached *via* a standard self-assembly of 3′-thiol derivatives of DNA onto a gold electrode. Sensors are prepared through binding of the redox-aptamer to the capture DNA whose length and sequence can be chosen to gain optimal signals for detection. Importantly, the present paper clearly demonstrates the feasibility of TISR in construction of electronic aptasensors for a large protein, exemplified by thrombin, as well as for a small molecule, ATP.



Scheme 1 A schematic representation of the TISR E-AB sensor. In the absence of target, the redox-tag appended onto the aptamer is near the electrode surface, thereby giving an intense electrochemical signal. With addition of target, *via* an aptamer structural switch, the redox-tagged aptamer strand is released from the surface and diffuses into solution, resulting in significant reduction of the signal.

The aptamer-capture DNA duplexes used for the ATP or the thrombin sensors are shown in Chart 1. Bis-anthraquinone-modified propanediol was used as a redox-tag that was introduced into the 5′-terminal position of anti-ATP (27-mer) and anti-thrombin aptamer (27-mer) using a phosphoramidite method.[15,16] 3′-Disufide-linked capture DNAs for ATP and thrombin sensors have complementary sequences; 15-mer to ATP and 21-mer to thrombin. The attachment of the capture DNAs onto electrodes was performed at room temperature

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† Electronic supplementary information (ESI) available: Materials and methods, electrochemical characterization, and selectivity of sensors. See DOI: 10.1039/b719089c

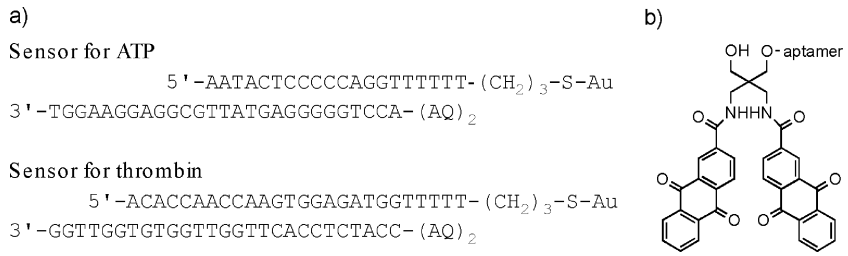

Chart 1 (a) Redox-tagged aptamer–DNA duplexes used for ATP or thrombin sensor. (b) The structure of the redox-tag used in this study.

by immersing (16 h) clean gold surfaces into the solutions of disulfide-liked capture DNAs (10 μM) in 100 mM Tris-HCl buffer (pH 7.4) containing 0.1 mM TCEP (tris-(2-carboxyethyl)phosphine hydrochloride). The masking of remaining surfaces was done by treatment of the DNA-modified gold electrodes with 2.5 mM mercaptopropanol in 10 mM Tris-HCl buffer. The duplex formation between the redox-tagged aptamer and the capture DNA was then performed with 5 μM aptamer solutions in 10 mM phosphate buffer (pH 7.0) containing 100 mM NaCl and 10 mM $MgCl_2$ at room temperature for 12 h. Electrochemical quantification[17] revealed that the amounts of the aptamer–DNA duplexes attached to the electrodes were 2–3 pmol cm^{-2}.

We first examined TISR in the detection of ATP. Fig. 1 shows the results from differential pulse voltammetry (DPV). In the absence of ATP, the redox-modified anti-ATP aptamer bound to the capture DNA shows the intense Faradic current due to the redox reaction of the appended anthraquinone. The observed current did not change in the absence of ligand for at least 12 h. Upon addition of 0.1 mM ATP, the current is significantly suppressed and the signal saturation (~65% decrease) was achieved within 80 min. More concentrated ATP (0.5 mM) resulted in further signal suppression up to 85%. Lower concentrated ligand (0.01 mM) gave the 10% signal suppression. The DPV peak current plotted against the logarithm of ATP concentration was linear. Therefore, the present E-AB sensor has the dynamic range detectable from ~0.01 mM to ~0.5 mM. Importantly, few changes were observed in the presence of 0.1 mM UTP and other nucleoside triphosphates. Our TISR approach thus provides a selective E-AB sensor for ATP.

Our TISR approach was further challenged by the detection of thrombin. Similar to the ATP sensor, the thrombin sensor displays an intense DPV signal in the absence of target (Fig. 2, panel a). Upon addition of 50 nM thrombin, the signal

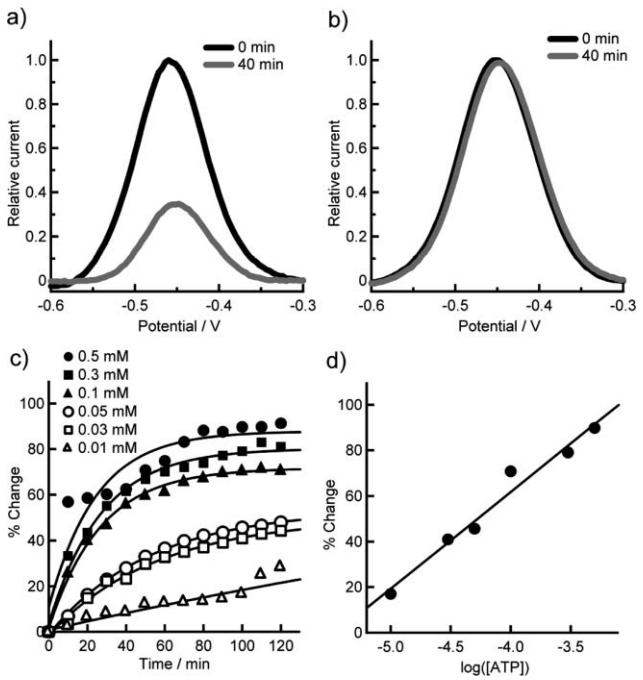

Fig. 1 (a) Differential pulse voltammetry (DPV) of the TISR E-AB sensor for ATP in the absence (black) or presence of 0.1 mM ATP (gray) after incubation for 40 min. (b) DPV in the absence (black) or presence of 0.1 mM UTP (gray) after incubation for 40 min. (c) Time dependent changes (%) in the current responses at different ATP concentrations. (d) Plot of % changes in the current responses *vs.* logarithm of ATP concentrations. Electrochemical measurements were carried out at room temperature in 20 mM Tris buffer (pH 7.6) containing 300 mM NaCl and 5 mM $MgCl_2$.

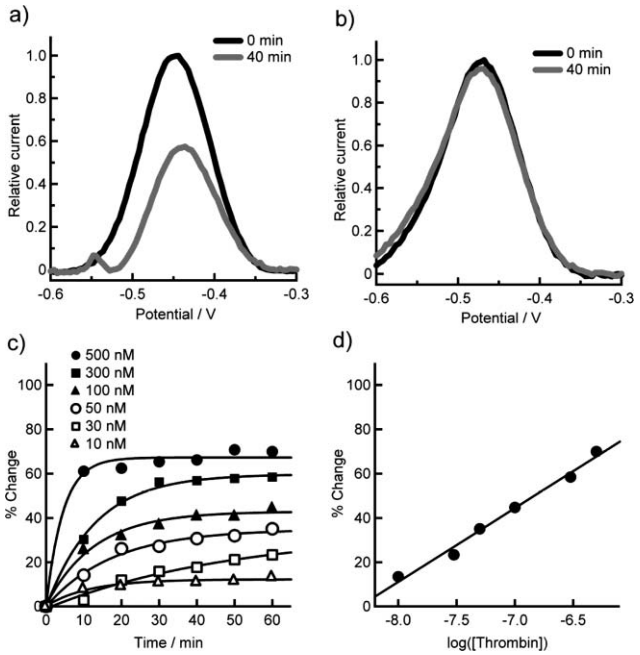

Fig. 2 (a) Differential pulse voltammetry (DPV) of the TISR E-AB sensor for thrombin in the absence (black) or the presence of 50 nM thrombin (gray) after incubation for 40 min. (b) DPV in the absence (black) or presence of 50 nM lysozyme (gray) after incubation for 40 min. (c) Time dependent changes (%) in the current responses at different thrombin concentrations. (d) Plot of % changes in the current responses *vs.* logarithm of thrombin concentrations. Electrochemical measurements were carried out at room temperature in 10 mM phosphate buffer (pH 7.6) containing 300 mM NaCl and 5 mM $MgCl_2$.

suppression was saturated within 30 min and a 40% signal decrease was observed. More concentrated thrombin (500 nM) further decreased the signal up to 65%. A 10% decrease of the signal was achieved with 10 nM thrombin. The % decrease in the peak current plotted against the logarithm of thrombin concentration was linear, showing that the thrombin sensor has a dynamic range over a 50-fold change in ligand concentration. Lysozyme (50 nM) suppressed the signal intensity less than 5%. The novel E-AB sensor is therefore both sensitive and selective for the target protein.

In summary, we have demonstrated that the target-induced strand release (TISR) approach is useful in the E-AB sensor development. The redox-tagged aptamers can be easily synthesized by the use of bis-anthraquinone compound. The preparation of sensors is simply performed by binding of redox-aptamers to capture DNA sequences bound on a gold electrode. The presence of target can be determined from the signal suppression due to the release of redox-tagged aptamer from the electrode into solution. With this strategy, the label-free, rapid, selective, and sensitive sensors particularly for a large protein are produced, although our method involves signal OFF architecture[10,14] rather than signal ON architecture.[12,13] The present TISR strategy should therefore be easily applicable to aptasensors that target a wide range of biomolecules.

This research is supported by grants from JST (CREST) and JSPS.

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