PAPER ELECTROCHEMICAL DEVICE FOR DETECTION OF DNA AND THROMBIN BY TARGET-INDUCED CONFORMATIONAL SWITCHING

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ABSTRACT
Here we report an electrochemical paper analytical device (PAD) for quantitative detection of oligonucleotides and proteins by employing the target-induced conformational switching of an electrochemically labeled aptamer. This simple and robust method is well matched to the equally simple and robust characteristics of the PAD platform. The demonstrated detection limits for DNA and thrombin are 30 nM and 16 nM, respectively, and the device-to-device reproducibility is better than ±10%. The PAD has a shelf life of at least 4 weeks, requires a sample volume of just 20 μL and no washing steps.

KEYWORDS: Biosensor, Electrochemistry, PAD, Aptamer

INTRODUCTION
Paper analytical devices (PADs) provide an inexpensive solution to environmental and medical diagnostic needs in both the developing and developed world [1]. Typically, analyte detection in PADs is through a color change [2,3], fluorescence [3], or electrochemical methods [4]. Of these, electrochemistry provides a good combination of simplicity, low power requirements, low limits of detection, and ease of quantitation [1]. In the present manuscript we have adapted an electrochemical detection method, originally reported by Plaxco and coworkers [5,6], to a paper platform. Surprisingly, the very simple and inexpensive paper device provides figures of merit nearly identical to those obtained using traditional three-electrode electrochemical cells. This is all the more remarkable, because the devices are highly reproducible, have long shelf lives, and require little user intervention.

The general approach is illustrated in Figure 1 for the two types of targets reported here: DNA and thrombin. As shown, the sensing mechanism is based on target-induced folding or unfolding of electrode-bound oligonucleotide probes that have a pendant redox reporter at the distal end and a thiol at the proximal end for easy attachment to a gold electrode. When the analyte binds, the probe undergoes a conformational change that alters the location of the redox reporter relative to the electrode. Depending on the nature of this change, the redox reporter may move closer to the electrode ("on" sensor) or further from the electrode ("off" sensor, as shown in Figure 1). This shift in conformation results in a change in faradaic current that is easily detected using either alternating current voltammetry (ACV) or square wave voltammetry (SWV).

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{sensor_schematic.png}
\caption{Electrochemical sensor schematic for detection of (a) DNA and (b) thrombin.}
\end{figure}

EXPERIMENTAL
Figure 2 illustrates the fabrication and operation of the paper electrochemical sensor, or "esensor" for short, used in this report. As shown in Figure 2a, the sensor consists of two pieces: a base layer and a slip
The base layer is fabricated by wax printing a pattern onto chromatography paper and then stencil printing three carbon electrodes onto the wax-patterned paper. A layer of Ag/AgCl paste is then painted on top of the reference electrode and Au is electroplated onto the working electrode (WE). Finally, the probe DNA is incubated on the Au WE for an optimized period of time. The slip layer is also fabricated on chromatography paper such that a hydrophobic wax frame surrounds the hydrophilic T-shaped section. The right side of Figure 2 illustrates the operation of the paper esensor. First, the channel is slipped down onto the three electrodes such that the horizontal paper section of the slip layer covers the three electrodes. Next, ~20 µL of buffer is added to the hole in the slip layer, which wets the paper and brings the three electrodes into electrochemical contact so that a background ACV or SWV can be obtained [4]. The channel is then slipped up to expose the WE through the hole in the flap of the base layer, and the sample is introduced. After a predetermined incubation period, the channel is slipped back into the measurement position and another ACV or SWV measurement is obtained.

**Figure 2:** A representation of how the esensor is (a-d) fabricated and (e-h) operated.

**RESULTS AND DISCUSSION**

In the case of ssDNA, as shown in Figure 1a, the sensing mechanism is based on target-induced unfolding of the electrode-bound stemloop that has a pendant methylene blue (MB) redox reporter. When the ssDNA binds, the probe undergoes a conformational change that moves the MB further from the electrode and therefore decreases the measured faradaic current. The ACV in Figure 3a shows that the initial peak current of 0.95 µA decreases by 44.8% after addition of 10.0 µM ssDNA. The average signal suppression and standard deviation for 10.0 µM target ssDNA are 50.8% and 4.6%, respectively (Figure 3b) which can be compared with that obtained by Plaxco and coworkers (~50%) using the same stemloop probe and ssDNA target, but a traditional electrochemical cell [5]. Surprisingly, after 4 weeks of dry storage under nitrogen the stemloop signal only decreased by 6%. We also examined the sensitivity to targets having one and two mismatched base pairs, as well as to a completely mismatched sequence, and the results (Figure 3b) indicated current suppressions of 17.7%, 14.1%, and 4.3%, respectively. A limit of detection (LOD) of 30 nM was determined from the dose-response curve in Figure 3c.

**Figure 3:** Detection of ssDNA using the device shown in Figure 2 and the conformational switching approach shown in Figure 1a. (a) Baseline-subtracted ACVs before and after the addition of target ssDNA. (b) Comparison of percent signal suppression for different numbers of ssDNA base mismatches. (c) Dose-response curve for ssDNA detection with a LOD of 30 nM.
To demonstrate the broad scope of this paper esensor design, we performed an assay for thrombin. In this case, the thrombin aptamer (Figure 1b) is a single DNA strand that exhibits a change in collision dynamics upon binding to thrombin. Figure 4a shows SWV results obtained before and after exposure to 2.0 µM thrombin. A LOD of 16 nM was determined using the dose response curve (Figure 4b). This value compares favorably to the dynamic range of a few nanomolar to several hundred nanomolar previously reported by Plaxco and coworkers [6].

Figure 4: Detection of thrombin using the device shown in Figure 2 and the conformational switching approach shown in Figure 1b. (a) SWV before and after the addition of thrombin. (b) Dose-response curve for detection of thrombin with a LOD of 16 nM.

CONCLUSION
To summarize, we have reported a paper-based esensor that is based on the principle of target-induced conformational switching. This is a remarkably simple and robust approach to biosensing, and consequently it is especially well-suited to the equally simple and robust characteristics of the type of PAD described here. The paper esensor design is easily adaptable to mass production, and the chemistry is sufficiently stable that the packaged esensor has a shelf life of at least 4 weeks. Moreover, the esensor is readily adaptable to other redox beacons, and hence it seems likely that multiplexing sensors on a single device is possible. Expanding the esensor design to include other targets and complex sample matrices, are our current areas of focus. The results of these experiments will be reported in due time.

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