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An Electronic, Aptamer-Based Small-Molecule Sensor for the Rapid, Label-Free Detection of Cocaine in Adulterated Samples and Biological Fluids

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Whereas spectroscopic and chromatographic techniques for the detection of small molecules have achieved impressive results,[1−4] these methods are generally slow and cumbersome, and thus the development of a general means for the real-time, electronic detection of such targets remains a compelling goal. Here we demonstrate that a previously described electronic aptamer-based sensing platform termed E-AB sensors[5] may meet this goal. We do so by building a label-free, electronic E-AB sensor for the detection of cocaine in complex, contaminant-ridden samples. The sensor is "signal-on", responds rapidly (seconds) and specifically to micromolar cocaine in blood serum, saliva, and adulterated samples, requires only inexpensive, off-the-shelf electronics, and regenerates via a brief, room temperature wash.

Cocaine serves as an ideal and representative target for testing new analytical techniques due to pressing needs for its rapid detection in law enforcement and clinical settings. We have fabricated an E-AB sensor for the electrochemical detection of this small molecule (Figure 1) using an aptamer previously engineered by Stojanovic et al.[6] The E-AB sensor is fabricated by self-assembly of the relevant methylene-blue (MB)-tagged aptamer on a ~1 mm^2 gold electrode via an alkanethiol group. In the absence of target, the aptamer is thought to remain partially unfolded, with only one of its three double-stranded stems intact.[6] In the presence of target, the aptamer presumably folds into the cocaine-binding three-way junction, altering electron transfer and increasing the observed reduction peak. Of note, the fold of the cocaine-binding aptamer is distinct from the aptamer employed in the first E-AB sensor,[5,7] thus providing evidence of the E-AB platform's generalizability.

To determine optimal redox tag and electrode attachment geometries, we have explored four different sensor architectures (Table 1). Upon aptamer immobilization and subsequent electrode passivation, aptamers A1, A3, and A4 give rise to large MB reduction peaks. In contrast, electrodes modified with A2 (containing only a three-carbon alkanethiol group) and passivated do not exhibit MB reduction in either the presence or absence of target, suggesting A2 is not adsorbed to the electrode. When immersed in cocaine-containing samples, the Faradaic currents produced by A1 and A4 increase significantly (Figure 2), presumably because the aptamers place their redox tags in close proximity to the electrode upon folding into their cocaine-binding configurations. Alternatively, the observed signal change could arise due to binding-induced modulation of the intercalation state of the C7-linked MB tag.[8] The signal produced by A3, in contrast, does not change in response to target (data not shown), suggesting that binding does not significantly affect electron transfer or that the internal modification reduces cocaine affinity.

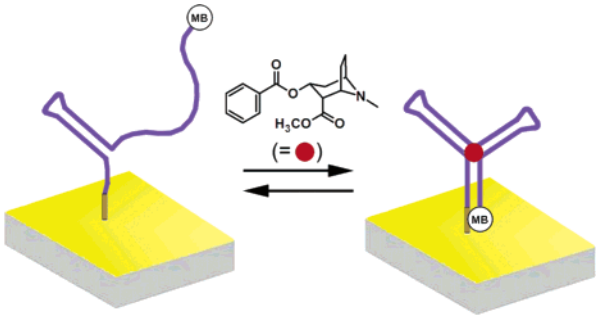

Figure 1. The electronic aptamer-based (E-AB) cocaine biosensor.

Table 1. Modified Aptamers[a]

	aptamer sequence (5′ → 3′)
A1	HSC6-GACAAGGAAAATCCTTCAATGAAGTGGGTC-MB
A2	MB-GACAAGGAAAATCCTTCAATGAAGTGGGTC-C3SH
A3	HSC6-GACAAGGAAAATCCTTCAATGAAGT(MB)GGGTC
A4	HSC6-AGACAAGGAAAATCCTTCAATGAAGTGGGTCG-MB

[a] Methylene blue (MB) redox tags are covalently attached via a seven-carbon linker to the termini, or an internal thymidine, as indicated.

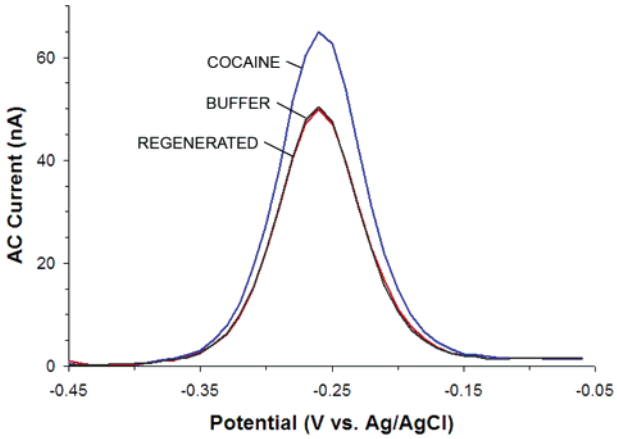

Figure 2. AC voltammograms of the A4 cocaine E-AB sensor (reduction peak) recorded in saline buffer (1 M sodium chloride, 10 mM potassium phosphate, pH 7) (black). Upon addition of 500 μM cocaine, a signal increase is observed. The sensor was then regenerated by immersing the electrode two times in room temperature buffer for 3 min (red).

E-AB sensors based on aptamers A1 and A4 display hyperbolic binding, with K_d of approximately 90 μM and detection limits of below 10 μM (Figure 3). The sensor equilibrates rapidly: we

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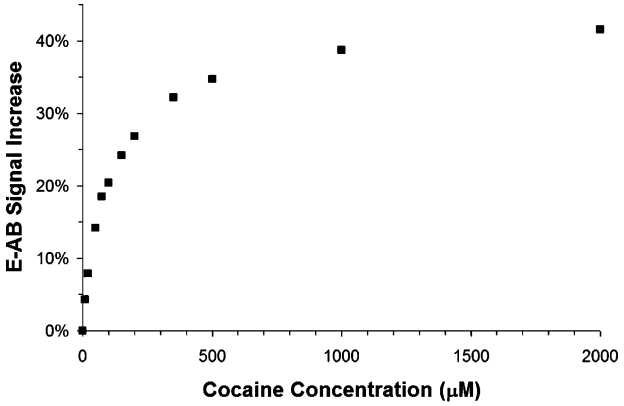

Figure 3. A dose-response curve for the A4 E-AB cocaine sensor in saline buffer.

Table 2. Cocaine Detection in Cutting and Masking Adulterants[a]

adulterant	Scott Test	E-AB signal
none	positive	+31.4%
equal mass flour	positive	+31.4%
equal mass sugar	positive	+31.5%
equal mass baking soda	positive	+31.2%
equal mass coffee	positive	+29.5%
equal mass mustard powder	positive	+30.7%
10X mass cobaltous thiocyanate	negative	+24.2%

[a] Cocaine mixed with cutting and masking agents was detected via the Scott test and, at ~200-fold lower concentration, the E-AB sensor. Scott Test results refer to the presence (positive) or absence (negative) of a color change upon addition of ~70 mg/mL of cocaine. The E-AB analysis, in contrast, was performed using a concentration of 150 μg/mL.

observe 97% of the total signal change within the first 80 s ACV scan and complete saturation in less than 4 min (data not shown).

To assess the E-AB sensor's ability to detect small molecules in complex, tainted samples, we tested sensors built with aptamers A1 and A4 for their ability to detect cocaine in the presence of biological fluids and other contaminants. We find that both sensors readily detected 500 μM cocaine in fetal calf serum or human saliva (both diluted 50% in buffered saline as an electrolyte), with signal increases of 12.3 and 16%, respectively. Upon immersion in cocaine-free buffered saline, the signal returned to the original value (within 2%). Similarly, the sensor is effectively impervious to the many agents employed to cut or mask cocaine[9] (Table 2). This includes cobaltous thiocyanate, a masking agent employed to obscure[10] the colorimetric Scott Test[11] commonly used by customs agents and law enforcement officials (Table 2). These results compare favorably with previously reported optical aptamer approaches,[6] which are generally limited by significant background fluorescence.[12,13]

Because of the rapid equilibration time of the sensor, simple immersion in target-free buffer for a few minutes leads to near complete regeneration (Figure 2). To demonstrate this and to monitor the reproducibility of the E-AB sensor, we repeated measurements in cycles of immersion in 500 μM cocaine, followed by two 3 min immersions in target-free buffer. We find that the coefficient of variability associated with these measurements is just 3%, and that, even after six rounds of use and regeneration, we recover the initial sensor signal to within 1%.

In summary, we have demonstrated a rapid, label-free, electronic method for the detection of small molecules. The E-AB sensor responds to its target in seconds and is easily regenerated via a brief room temperature wash. Moreover, because the sensor is based on a target-induced conformational change (rather than a less specific signaling mechanism, such as adsorbed mass or charge), it is unaffected by nonspecific contaminants, as evidenced by our detection of cocaine in blood serum, saliva, and other complex, contaminant-ridden samples. Given recent advances in aptamer selection protocols, which yield aptamers against targets ranging from proteins[14,15] to small molecules,[16] new techniques developed to specifically select aptamers with signal transduction properties,[17−19] and the observation that completely distinct structural classes of aptamers can be employed,[5,7] it appears that E-AB sensors may provide a means for the convenient, specific detection of a wide range of targets. Finally, given the small electrode size and label-free nature of the approach, the generation of E-AB biosensing elements may allow the development of densely packed, multi-analyte sensing arrays,[20] for the simultaneous detection of numerous compounds in environmental, clinical, or civil defense applications.

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Supporting Information Available: Materials and methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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