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Analyte-induced formation of partial duplexes for the preparation of a label-free electrochemiluminescent aptasensor†

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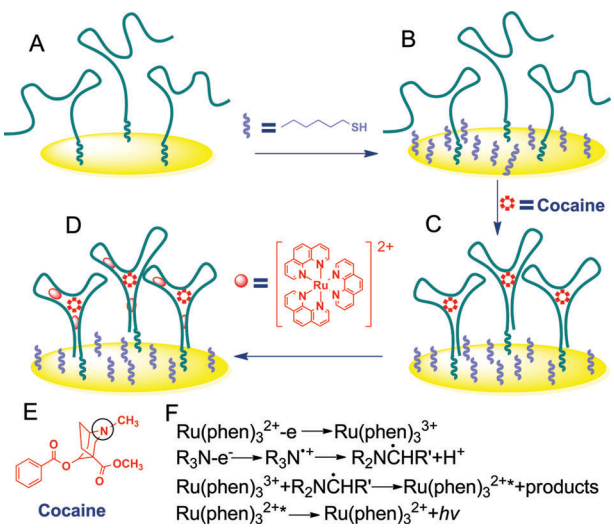
Analyte-induced formation of partial duplexes was used for biosensor development with cocaine as a model. The cocaine–aptamer interaction resulted in formation of a partial double strand section in the aptamer, where $Ru(phen)_3^{2+}$ was intercalated for electrochemiluminescent analysis of cocaine.

DNA aptamers are single-stranded nucleic acids that provide an alternative to antibodies in heterogeneous assays.[1] During recognition of its target molecule, an aptamer folds into a three-dimensional complex and this can be exploited for the construction of aptamer-based biosensors (aptasensors).[2] Folding of an aptamer that is labeled with a signal molecule produces a change in the signal on target recognition. This change is used to quantify the target with different detection techniques, such as electrochemiluminescence (ECL),[3] electrochemistry,[4] fluorescence,[5] and colorimetric assay.[6] Plaxco *et al.*[7] developed various electrochemical aptasensors using aptamer folding. However, a signal of approximately 30% of the saturated target level was observed even in the absence of the target because ss-DNA collapsed on the electrode rather than standing upright.[7a] Moreover, the chemical labeling procedure was complex, time-consuming, and labor-intensive.[6a] Therefore, it is critical that a strategy with a simplified procedure and a decreased background signal is developed for aptasensors.[7,8]

During molecular recognition, formation of a partial duplex may be induced in the aptamer.[9] Helical double stranded DNA (ds-DNA) can intercalate some small molecules into its grooves with high affinity.[10] Therefore, the formed partial duplex in the aptamer could be exploited for the development of label-free aptasensors based on the ability to intercalate probe molecules.[10] Because the signal is produced from the probe intercalated into the induced duplex section, this method would have a low background signal and reduced false positives compared to the method using folding of labeled aptamers. Analyte-induced formation of the duplex is also simpler than the artificial formation strategy.[10a,b]

In this study, the partial duplexes formed in an aptamer during molecular recognition were used to intercalate a $Ru(phen)_3^{2+}$ probe for ECL analysis of cocaine. The anti-cocaine aptamer was first immobilized onto a gold electrode *via* a gold–thiol bond (Scheme 1A and B). Binding of cocaine to its aptamer resulted in formation of the duplexes (Scheme 1C).[7c] The $Ru(phen)_3^{2+}$ probe was then intercalated into the duplex sections (Scheme 1D).[10a,b] ECL emission corresponding to the concentration of cocaine was observed when a constant potential was applied to the modified electrode. Because the probe was introduced within the double strand sections formed during the recognition process, the background signal was lower than that observed with previous aptasensors.[7]

Formation of the duplex sections during aptamer recognition of cocaine and the excitation of $Ru(phen)_3^{2+}$ ECL were critical for the aptasensor function. Sun *et al.*[3b] obtained the binding constant of $4.6 \pm 0.3 \times 10^9$ mol L^{-1} between cocaine and its aptamer, but the dissociation constant of 200 μmol L^{-1}



Scheme 1 Schematic of the label-free aptasensor with the analyte-induced formation of duplex sections for probe intercalation. (A) Self-assembly immobilization of the anti-cocaine aptamer on the gold electrode; (B) blocking of the active site with 6-mercapto-1-hexanol; (C) cocaine induced formation of the duplexes; (D) intercalation of $Ru(phen)_3^{2+}$ into the formed double strand sections. (E) The molecular structure of cocaine. (F) The $Ru(phen)_3^{2+}$ ECL excitation mechanism of cocaine as a tertiary amine-containing molecule.[11]

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was obtained by the other researchers.[1] When interacting with cocaine, the aptamer formed a T-junction with two duplex sections as shown in Scheme 1C.[1] The ECL probe, $Ru(phen)_3^{2+}$, was intercalated in these sections (Scheme 1D). The tertiary amine group (Scheme 1E and R_3N in Scheme 1F) in the cocaine molecule was involved in the ECL reaction. $Ru(phen)_3^{2+}$ was oxidized electrochemically to $Ru(phen)_3^{3+}$, and cocaine was then oxidized in reaction with $Ru(phen)_3^{3+}$ and formed the tertiary amine radical. This active radical species reacted with the $Ru(phen)_3^{3+}$ to produce the excited state $Ru(phen)_3^{2+*}$ for ECL emission.[11] Thus, cocaine acts as the analyte, the species inducing formation of duplexes, and the co-reactant for ECL emission. As a "turn-on" detection system, the aptasensor does not suffer from the false positive and limited signal range of "turn-off" systems.[12]

Fig. S1 and S2 (ESI†) present the results for optimization of the aptasensor for cocaine. No anodic current was observed at the anti-cocaine aptamer-modified electrode. Once cocaine interacted with the immobilized aptamer, an anodic peak related to the oxidation of cocaine appeared at approximately 0.82 V (Fig. S4, ESI†). The peak potential was similar to that for the oxidation of tripropylamine (TPA).[10a,b] After the modified electrode was incubated in $Ru(phen)_3^{2+}$ solution, a peak for oxidation of $Ru(phen)_3^{2+}$ appeared at 1.02 V. The catalytic effect of $Ru(phen)_3^{2+}$ increased the oxidation current. As shown in Fig. 1, anodic peaks corresponding to the oxidation of cocaine (1st peak) and $Ru(phen)_3^{2+}$ (2nd peak) were observed. The linear relationship between the peak currents and the potential scan rates for the two species (Fig. 1B and C) shows that their electrochemistry is surface-controlled. These results indicate the cocaine-induced formation of the duplex and intercalation of the $Ru(phen)_3^{2+}$ probe.

To further validate the cocaine-induced formation of the duplexes, the ECL emission was monitored after intercalation of $Ru(phen)_3^{2+}$ for the aptamer-modified electrode with and without cocaine. Without cocaine, a low ECL emission (282 counts) was observed (Fig. 2Aa) even though 20 mmol L^{-1} TPA was present in the electrolyte and DNA can improve TPA oxidation.[10a,b] However, the ECL emission increased to

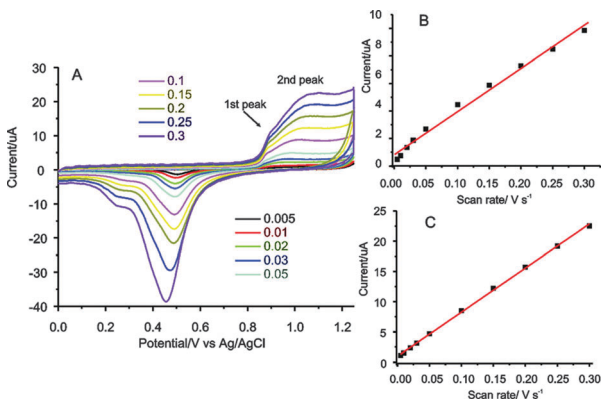

Fig. 1 (A) Cyclic voltammograms of the anti-cocaine aptamer-modified Au electrode after incubation with cocaine and intercalation of $Ru(phen)_3^{2+}$ at different scan rates. (B) Relationship between the 1st anodic peak currents and the scan rates. (C) Relationship between the 2nd anodic peak currents and scan rates. Electrolyte: 0.1 mol L^{-1} phosphate buffer solution (pH 7.5).

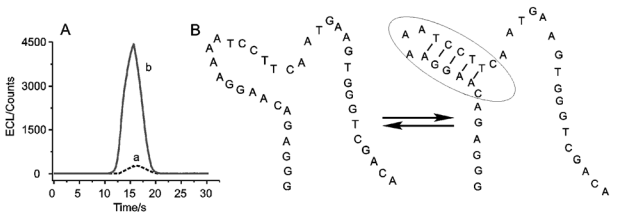

Fig. 2 Validation of the analyte-induced formation of duplexes. (A) ECL emission of (a) the electrode modified anti-cocaine aptamer after intercalation of $Ru(phen)_3^{2+}$ and (b) the electrode modified aptamer interaction with cocaine for the intercalation of $Ru(phen)_3^{2+}$; (B) the equilibrium between different forms of the anti-cocaine aptamer.[7a,b] The conditions were 0.1 mol L^{-1} phosphate buffer solution (pH 7.5) containing 20 mmol L^{-1} TPA, and a scan rate of 50 mV s^{-1}.

4486 counts with 10 pmol L^{-1} cocaine interacting to its aptamer (Fig. 2Ab). This difference in ECL emission was attributed to cocaine-induced formation of the duplexes for intercalation of $Ru(phen)_3^{2+}$. Every four base pairs (bps) can intercalate one $Ru(phen)_3^{2+}$ molecule,[10] so two $Ru(phen)_3^{2+}$ molecules can be intercalated into the formed duplexes. The aptamer may fold to form a partial duplex, which exists in equilibrium with the free form (Fig. 2B).[7a,b] However, the difference in ECL emission with and without cocaine indicates that most of the aptamer existed in the free form under the present conditions.

The specificity of the induced aptamer conformation changes was validated with the ECL response upon interaction with cocaine and with the non-target molecules, including 0.1 mmol L^{-1} glucose, L-histidine, and L-arginine. These non-target species were selected because they are frequently present in biological samples. The ECL intensity produced with the three non-target species (Fig. 3b–d) was minimal compared to that produced with 10 pmol L^{-1} cocaine (152 counts, Fig. 3a). No evidence was found for binding of the non-target species to the aptamer, which indicates that these species were unable to induce formation of the duplex for intercalation of the $Ru(phen)_3^{2+}$ probe, showing the duplex formation specific to cocaine. The ECL intensity produced with 10 pmol L^{-1} cocaine reached its maximum within 0.3 s once the potential reached 1.25 V *vs.* Ag/AgCl (inset of Fig. 3). Therefore, the ECL emission occurred by a fast electrochemical process. The observation of ECL emission with 10 pmol L^{-1} cocaine, but not with the non-target species (Fig. 3), shows that the aptasensor has both high sensitivity and selectivity.

Fig. S5 (ESI†) shows the profile between the ECL emission and the cocaine concentration with a linear range of three orders

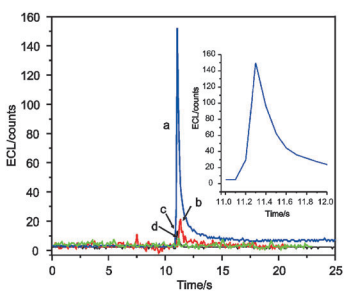

Fig. 3 The ECL response of the aptasensor for (a) 10 pmol L^{-1} cocaine, and 0.1 mmol L^{-1} level (b) glucose, (c) L-histidine, and (d) L-arginine in 0.1 mol L^{-1} phosphate buffer solution (pH 7.5) at a constant potential of +1.25 V *vs.* Ag/AgCl. Inset: the amplification of profile (a).

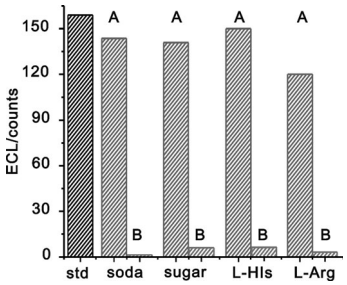

Fig. 4 Selectivity of the ECL aptasensor with cocaine-induced formation of ds-DNA for probe intercalation. (A) 0.1 mmol L^{-1} interfering species and 10 pmol L^{-1} cocaine and (B) only 0.1 mmol L^{-1} interfering species.

of magnitude (R^2 = 0.997). A detection limit of 0.2 pmol L^{-1} was achieved because of the almost zero background signal and the high sensitivity of the ECL response. An absolute mass detection limit of 2.0 fmol was obtained with 10 μL of sample. This detection limit is more than two orders of magnitude lower than that obtained with previous aptasensors (Table S1, ESI†). By comparison, an earlier ECL sensor for cocaine by double covalent coupling gave a detection limit of 10 pmol L^{-1}.[3b] In comparison with the earlier sensors,[1,4–6] the present sensor is simpler to produce and more sensitive for cocaine. The sensor also gives a good reproducibility with a relative standard deviation (RSD, n = 6) of 4.9% (Fig. S6, ESI†).

To check the selectivity and practicality of the aptasensor for cocaine, the results were compared for a series of samples containing 10 pmol L^{-1} cocaine and/or 0.1 mmol L^{-1} soda, glucose, L-histidine, and L-arginine (Fig. 4). Only cocaine produced a large ECL response, and all the interfering species gave negligible emission even though their concentrations were 10 000-fold higher than that of cocaine. With the mixtures of cocaine with soda, glucose, L-histine, or L-arginine, the recovery was 91.1%, 89.1%, 95.0%, and 75.8% of that of cocaine alone, respectively. These results indicate that the aptasensor is specific to cocaine and the other species and the matrix do not interfere with the detection.

Because of its high selectivity and sensitivity the aptasensor could be applied to the detection of cocaine in human urine and plasma samples. No cocaine was detected in the urine and plasma samples. For samples spiked with cocaine at different levels, the recoveries ranged from 79 to 96% (Table S2, ESI†). These results indicate that the proposed aptasensor could be used to detect cocaine in biological samples.

In conclusion, interaction of a target molecule with an aptamer can induce formation of a duplex in the aptamer, which can be used to intercalate probe molecules. This was exploited to produce a direct, label-free, specific, and sensitive ECL method with a detection limit (0.2 pmol L^{-1}) for cocaine. This aptasensor had high specificity for cocaine over other species. Compared to other aptasensors with more than one DNA strand,[10a,b,13] this method was simple and used only the aptamer strand for the label-free detection. Although some previous studies[7a] have confirmed that the conformational changes from folding occur when an aptamer recognizes its target, this work provides evidence for the process of this conformation change, the formation of the duplex, during recognition. Most of the aptamers will form duplex sections to recognize their targets, which means that this strategy could be used to develop the aptasensors for other targets.[14]

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