A chronocoulometric aptamer sensor for adenosine monophosphate†

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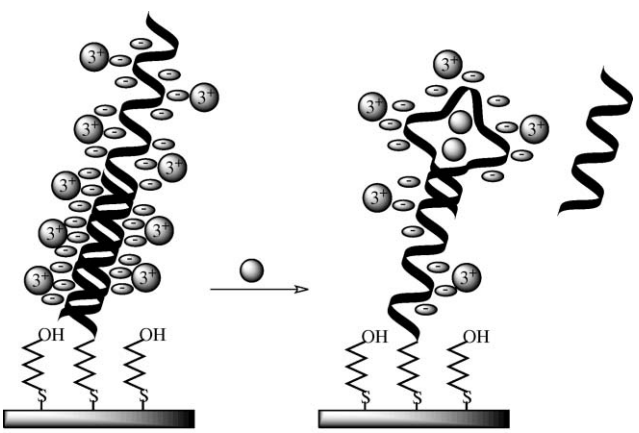
The selective recognition of adenosine monophosphate by a half-duplex aptamer-modified electrode leads to a simple chronocoulometric aptasensor based on the changes in surface charges.

Aptamers,[1,2] selected from combinatorial libraries using SELEX (systemic evolution of ligands by exponential enrichment), are oligonucleotides (DNA or RNA) which can bind with high affinity and specificity to a wide range of target molecules, including drugs, proteins and other organic or inorganic molecules. Because of their specific binding abilities, aptamers are starting to appear in biosensor applications, such as optical[3,4] and electrochemical biosensors (E-AB sensor).[5] For electrochemical detections, there are some redox-labeled aptamers which have been used for the voltammetric detections of thrombin,[6] cocaine[7] and potassium ions.[8] Currently, several efforts are focused on label-free E-AB sensors since the covalent labeling of an oligonucleotide is time-consuming, involving complicated synthetic procedures. For example, a novel label-free impedance strategy was used to analyze the aptamer–thrombin recognition.[9] A label-free electrochemical detection of thrombin based on a ferrocene-bearing cationic polythiophene and an aptamer has been reported.[10] A selective label-free potentiometric sensor of small molecules based on an ion-selective field-effect transistor has also been developed.[11]

It has been reported that a $[Ru(NH_3)_6]^{3+}$ (RuHex)–DNA–electrode system can be used to generate a significantly more intense signal in chronocoulometry (CC) than that in voltammetry.[12] Early studies employed the CC technique to quantify DNA surface density[13] and monitor DNA hybridization.[14] The principle is that RuHex cations can exchange with native counterions associated with the nucleotide phosphate residues of the probe. The amount of redox marker "electrostatically trapped" at the DNA-modified electrode is then determined using CC.[13]

In this work, based on a similar concept, we fabricated a chronocoulometric aptamer sensor for adenosine monophosphate (AMP). The electroactive complex, RuHex, which can bind to anionic phosphates of DNA strands completely through electrostatic interactions, serves as a signaling transducer. The successful protocol provides an example of using electrostatic interactions between aptamers and redox mediators for fabricating aptamer sensors. Here, the solution of modified aptamer (**1**) for AMP and the complementary short oligonucleic acid (**2**)[3,11] were first mixed in the buffers in equal mole quantities to create a half-duplex, and then the half-duplex aptamers were immobilized onto a gold electrode surface *via* thiol–Au interactions. The surface density of the duplex was determined to be 4.8×10^{12} molecules per cm^2 using the procedure of Steel *et al.* (see supplementary information†),[13] which is in good agreement with that found previously.[13,14] When the modified electrode was incubated in the AMP solutions, the aptamers made allosteric changes to bind AMP. As a result, only five base pairs are left to hybridize with (**2**), which is unstable at room temperature.[3] Therefore, the short complementary nucleic acids (**2**) were released together with the RuHex that was electrostatically binding to them, resulting in a lower chronocoulometric signal for the RuHex confined on the electrode surface (Scheme 1).

Previous studies have revealed that RuHex has a unique electroactive behavior on the surface of a DNA–mercaptohexanol (MCH) modified electrode.[12,14] Two pairs of well-defined peaks can be observed using cyclic voltammetry when the DNA–MCH modified electrode is immersed in a solution containing RuHex at a low ionic strength. One pair of peaks, similar to that observed on a MCH-modified electrode, is attributed to the redox reaction of RuHex diffused to the MCH portion in the mixed DNA–MCH monolayers. The other pair of peaks at about −0.3 V is characteristic of a surface-confined redox process which reflects the amount of DNA strands localized at the electrode surface. The



Scheme 1 A schematic representation of the chronocoulometric aptamer sensor for AMP.

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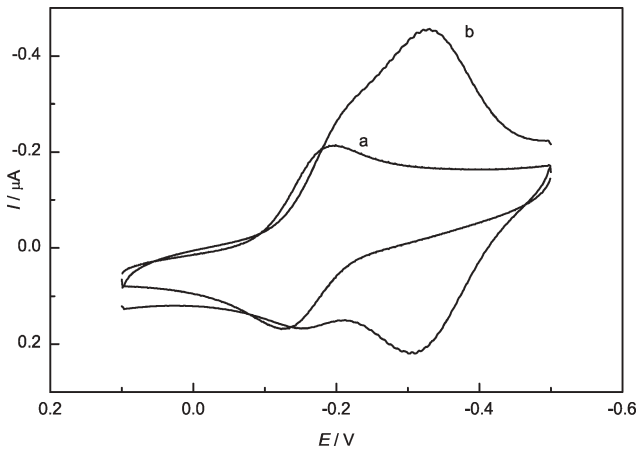

Fig. 1 Cyclic voltammograms of gold electrodes modified with (a) mercaptohexanol and (b) half-duplex–mercaptohexanol in 10 mM Tris buffer (pH 7.4) with 50 μM RuHex. Scan rate: 50 mV s^{-1}.

similar voltammograms in Fig. 1 indicate that the half-duplex has definitely been modified onto the gold electrode surface. In this work, the CC technique was employed for detection of AMP in solutions. Fig. 2 shows typical chronocoulometric curves of the duplex-confined electrode before (a) and after (b–f) incubation at different concentrations of the target. It is clear that the amount of surface charge is decreased with an increase in the AMP concentration. The dynamic range of the chronocoulometric aptamer sensor (Fig. 3a) covers a large variation of AMP concentrations from 0.1 μM to 1 mM. The detection limit is below 0.1 μM with a response time of less than 10 min (see the inset in Fig. 3). Although the sensitivity of the sensor is not as good as that of fluorescence-based detection methods,[4,15] it is much better than the colorimetric[3] and other electrical AMP aptasensors.[11] In a control experiment, the chronocoulometric signals of the electrode had little changes after treatment with different concentrations of cytidine monophosphate (CMP) (Fig. 3b), indicating the aptamer is incapable of binding molecules similar to AMP in structure. Therefore, the aptamer sensor possesses high specificity due to the inherent specificity of the aptamer towards its

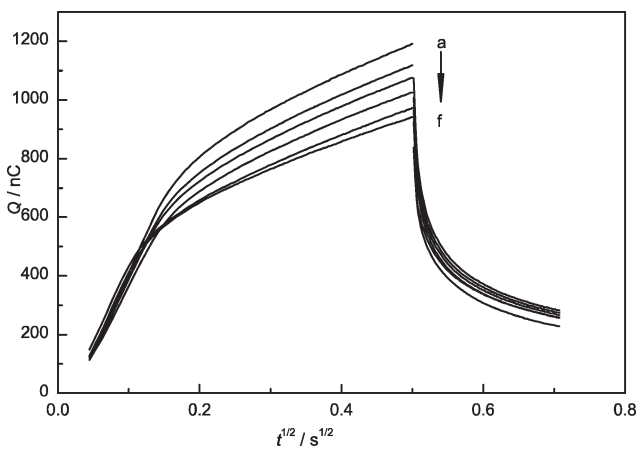

Fig. 2 Chronocoulometric curves for half-duplex modified electrodes upon addition of different concentrations of AMP: (a) 0 M, (b) 10^{-7} M, (c) 10^{-6} M, (d) 10^{-5} M, (e) 10^{-4} M and (f) 10^{-3} M. The solution is 10 mM Tris buffer (pH 7.4) containing 50 μM RuHex.

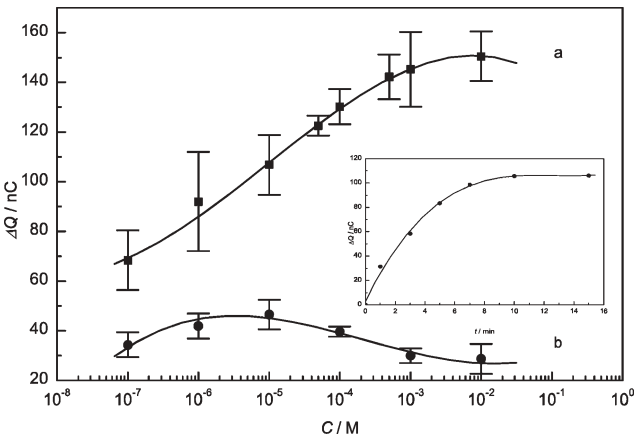

Fig. 3 Calibration curves: (a) AMP and (b) CMP. The data were obtained from three independent measurements and the error bars indicate the standard deviations. Inset: the time-dependent response of the duplex-modified electrodes upon analyzing AMP, 10^{-3} M.

target. The practical performance of the sensor has been examined by analyzing AMP spiked in a working matrix containing 1 mM CMP and GMP (guanosine monophosphate). The sensitivity and selectivity of the AMP sensor are both unaffected by the presence of those AMP analogues, indicating that the aptasensor might be a promising tool for AMP assays in real samples.

It has been demonstrated that the presence of potassium ions can induce the folding of a G-rich aptamer from a loose random coil into a compact G-quadruplex in a manner similar to its target.[16] Based on this principle, a G-rich thrombin aptamer which can form a chair-type quadruplex structure upon binding to thrombin was employed to construct K^+ aptasensors with low detection limits (below 1 mM).[8,17] The structure of the aptamer for AMP, composed of two stacked G-quartets, appears to be remarkably similar to that of the aptamer for thrombin.[18] K^+ ions may have a great effect on the CC aptamer sensor for AMP. When the half-duplex modified electrode is exposed to a solution of K^+, the CC signals decrease significantly (see Fig. S2 in the supplementary information†). The aptasensor would be better used to analyze AMP solutions in the absence of K^+ ions.

The sensor relies on the electrostatic binding of RuHex to the DNA phosphate sites, and thus the ionic strength of the RuHex solutions should play a key role in the performance of the sensor. When NaCl is added to the RuHex solution, the current of the peaks corresponding to bound RuHex decreases apparently with the increase in NaCl concentration. And the pair of peaks almost disappears when the concentration of NaCl reaches 0.1 M. In contrast, the diffusion-controlled peaks of RuHex increase. In addition, the CC signal of the sensor reduces sharply (see Fig. S3 in the supplementary information†), which is consistent with the voltammetric responses. The results show that the cations in the solution can compete with RuHex for binding to the anionic DNA backbone. The increase in ionic strength will weaken the interactions between RuHex and DNA, leading to a decrease in the amount of adsorbed RuHex at the DNA-modified electrode. Therefore, it is necessary to maintain low ionic strength conditions.

In summary, we have demonstrated that the half-duplex aptamer can be easily immobilized on a gold electrode and can change its conformation to bind its target, with the short

complementary oligonucleic acid being released. The chronocoulometric technique was first employed in this type of electrochemical aptasensor for detecting the changes in the surface charges *via* RuHex redox in solution. This chronocoulometric aptasensor, exhibiting high sensitivity and selectivity, provides a new way for analysis of other small molecules. However, there are still some challenges left. For instance, more sensitive and reusable aptasensors would be preferred. Work on this track is being undertaken in our lab.

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