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The ion gating effect: using a change in flexibility to allow label free electrochemical detection of DNA hybridisation†

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A label free electrochemical method of detecting DNA hybridisation is presented based on the change in flexibility between a single strand of DNA and a duplex causing an ion-gating effect where hybridisation opens up the electrode to access of ions.

DNA biosensors are attracting enormous interest as a simple method of detecting specific sequences of DNA.[1] The detection of specific sequences of DNA is usually performed by hybridisation with the complementary sequence. Therefore a DNA biosensor involves the immobilisation of the detection sequence of DNA, the *probe*, onto a surface and some method of transducing the hybridisation event. Hybridisation then causes a response in a signal transducer. Electrochemical transduction of DNA hybridisation is becoming increasingly popular and has recently been reviewed.[2,3] The vast majority of the electrochemical approaches to the detection of DNA require some sort of label, a redox active molecule, which gives a difference in response between the single strand immobilised on the transducer surface and when hybridised with the complementary sequence. Regardless of whether the redox label is attached to the probe sequence,[4] a reporter sequence[5] or added in solution[6,7] its presence complicates either the fabrication or the use of the DNA biosensor. There have however been a few attempts to develop electrochemical DNA biosensors which are label-free[8–11] which either rely on direct electrochemistry of the target DNA[9] or a change in the electrical properties of the interface as a consequence of the DNA hybridisation.[8,10,11] Here we present a novel label-free electrochemical method for the detection of DNA hybridisation at a self-assembled monolayer (SAM) modified electrode surface. This method is based on the change in flexibility of the DNA upon hybridisation opening the interface to the passage of ions to the electrode surface.

To achieve high hybridisation efficiencies the DNA recognition interface was made in a similar way to Levicky *et al.*[12] *via* the formation of a mixed SAM of thiolated DNA and an alcohol terminated alkanethiol as a diluent. The purpose of the diluent was to prevent the bases of the DNA complexing to the gold, so that the probe was end-point immobilised and accessible for hybridisation. In this study the probe DNA had a mercaptopropyl linker attached to the 3′ end of the probe sequence of DNA, sequence GGG GCA CTC CCT CAC AAC CT (Genset Oligos Pacific Pty Ltd, Sydney, Australia) and a diluent of mercaptoethanol, MCE (Sigma, Sydney, Australia). A polycrystalline gold surface, cleaned and characterised as described previously,[13] was placed in a 1.0 μM oligonucleotide solution in sterilised 1 M KH_2PO_4 at pH 4.5 for $1\frac{1}{2}$ h followed by rinsing in pH 7.0 phosphate buffer. The electrodes were then placed in a 1 mM MCE solution in Milli Q water for 30 min followed by rinsing to give a ss-DNA modified electrode. A cyclic voltammogram of the modified electrode performed in 0.05 M phosphate with 0.3 M NaCl (pH 7.0) with an Ag/AgCl reference electrode (BAS, Lafayette, IN, USA) and a platinum flag auxiliary electrode is shown in Fig. 1A. As can be seen the CV is relatively featureless. No change in this electrochemical behaviour was observed even three days after the electrode preparation. Hybridisation to the complementary sequence was performed by placing the ss-DNA modified electrode in a 1 μM solution of the complementary sequence in a buffer of 1 M NaCl and 10 mM Trisma at pH 7.0 for at least 2 h followed by rinsing. Returning the electrode to a solution of 0.05 M phosphate with 0.3 M NaCl (pH 7.0) gave the CV in Fig. 1B. Prominent oxidative electrochemistry is observed at +0.3 V with a reduction peak at +0.1 V also appearing. Denaturing the duplex back to only ss-DNA immobilised onto the electrode results in the diminution of the oxidation and reduction peaks back to levels similar to those observed prior to formation of the duplex. Repeating this process of hybridisation and denaturing with the associated appearance and disappearance of the redox peaks can be continued for at least three cycles. If the ss-DNA modified electrode is exposed to a non-complementary sequence in exactly the same way there is no apparent change in electrochemistry (see ESI†). This observation very clearly shows that the DNA modified electrode allows selective recognition of the DNA sequences with label free transduction. Further evidence for the observed electrochemistry to be due to DNA hybridisation is presented in Fig. 2, which shows a calibration curve of the magnitude of the oxidation peak relative to the concentration of target DNA.

The important question is what is the source of this electrochemistry since there is no obvious electroactive species in the sample or on the interface. Some significant observations have been made. Firstly, the electrochemistry is observed in a SAM of MCE alone (see ESI†) which suggests it is related to the gold–thiol bond. Such a conclusion is strengthened by previous work by Jiang *et al.*[14] where similar electrochemistry was observed for a variety of different short chain alkanethiol modified electrodes with different terminal groups. Thus the electrochemistry at +0.3 V appears to be an oxidation of the SAM. Oxidation of the gold–thiolate bond with subsequent desorption[15] appears a likely candidate but there are two

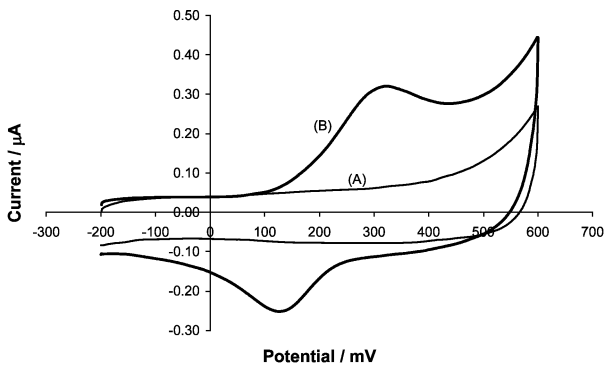

Fig. 1 (A) CV of the ss-DNA modified electrode performed in 0.05 M phosphate and 0.3 M NaCl (pH 7.0) with an Ag/AgCl reference electrode and a platinum flag auxiliary electrode. (B) CV of the same modified electrode after 2 h hybridisation of the probe DNA to a complementary sequence in solution.

† Electronic supplementary information (ESI) available: CVs: ss-DNA modified electrode exposed to a non-complementary sequence; MCE-modified gold electrode. See http://www.rsc.org/suppdata/cc/b3/b305798b/

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important pieces of evidence which suggest this is not the case. Firstly, with dodecanethiol the oxidative adsorption occurs at potentials positive of 1 V *vs.* Ag/AgCl.[13] Secondly, the oxidation peak was not present with fresh SAMs, taking an hour or more to appear in the CV. These two observations indicate a slow process is occurring which gives a weaker bond with gold than a thiol. This slow process is most likely oxidation as alkanethiol SAMs have been reported to oxidise to alkylsulfinate and alkylsulfonate species upon oxygen reaching the metal surface.[16,17] The rate of oxidation decreases with the length of the alkyl chain and any hydrogen bonding occurring between the terminal groups of the SAM.[17] We therefore propose that the observed electrochemistry is due to the oxidation of the sulfinate terminated SAM (which is less strongly adsorbed to the gold than a thiol[18]) to a sulfonate followed by reduction back to the sulfinate during the cathodic scan. Evidence for this oxidation process comes from a surface enhanced Raman spectroscopy study by Garrell *et al.*,[18] who observed the oxidation of a benzylsulfinate SAM to a sulfonate and back again as the potential of the underlying electrode was scanned anodically and then back cathodically. A more detailed investigation of the proposed electrochemistry will be described elsewhere.[19]

So how does the DNA influence the oxidation and reduction of the SAM? The presence of ss-DNA appears to protect the SAM from the oxidation of the sulfinate to a sulfonate. Either this is because the MCE has not been oxidised (recall that SAMs terminated with polar terminal groups were more resistant to oxidation[17]) or the sulfinate is prevented from oxidising to a sulfonate. We favour the latter for two reasons. Firstly even when allowing the ss-DNA modified electrode to sit in the air for 3 days, ample time for the SAM oxidation, no redox peaks are observed. Secondly, if the DNA duplexes are denatured, the redox peaks disappear, although there is some shift in the background current. The appearance and disappearance of the peaks can be repeated for more than three cycles of hybridisation and denaturing of the duplex. What is clear from the CV in Fig. 1 is that upon hybridisation the capacitance of the interface increases which suggests that hybridisation gives a more open interface which is not the case when the interface is exposed to non-complementary DNA. This change in capacitance can be related to the change in structure of the DNA. With the 20-mer used in this study ss-DNA is a flexible molecule while ds-DNA acts as a rigid rod.[12] Prior to hybridisation the ss-DNA can lie across the interface (see Fig. 3) and prevent ions from reaching the gold surface. In contrast upon hybridisation the ds-DNA is a rigid rod standing up off the surface, opening up the interface to ions, which can access the electrode at defect sites in the SAM, and thus an increase in capacitance is observed. This *ion-gating effect* then allows the electrochemistry to occur. As the oxidation and reduction peaks

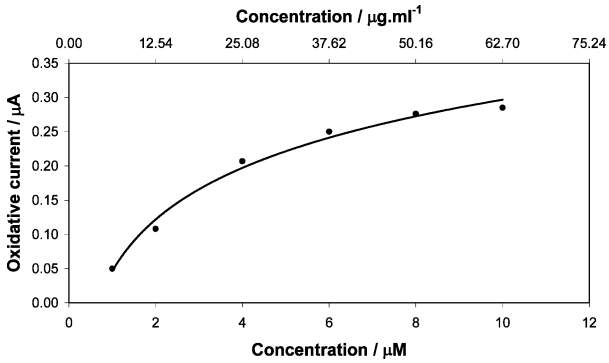

Fig. 2 Calibration curve of the magnitude of the oxidative peak relative to the concentration of the target DNA. Measurement conditions are as in Fig. 1. The hybridisation was for 2 h.

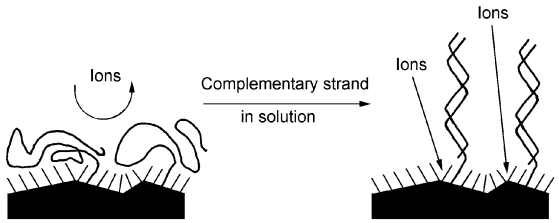

Fig. 3 A schematic diagram of the proposed process occurring at the electrode surface. The ss-DNA is flexible and lies across the interface, preventing ions from reaching the gold electrode. After hybridisation the rigid ds-DNA stands up off the surface, opening up the interface to ions, and thus producing an electrochemical signal.

diminish with increasing numbers of scans the oxidation appears to be an oxidative desorption of the SAM. For such an oxidation to occur, and the resultant sulfonate to diffuse into solution, requires cations to be able to access the electrode surface to maintain electrical neutrality. Therefore we propose the ss-DNA prevents access of cations to the electrode surface and no electrochemistry is observed. In contrast hybridisation opens the interface, allowing cations to access the surface and the electrochemical oxidation to occur. The decrease of the oxidation and reduction peaks back to the levels prior to hybridisation supports this proposition.

In conclusion we propose that label free detection of DNA hybridisation can be achieved using an ion-gating effect at an electrode modified with thiolated DNA and an MCE diluent layer. The oxidative desorption of the oxidised SAM is modulated by the change in structure of DNA upon hybridisation from flexible ss-DNA which blocks access of ions to the surface to rigid ds-DNA which opens the interface to cations.

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