Electrochemical SNPs detection using an abasic site-containing DNA on a gold electrode

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An abasic site-containing DNA combined with lumiflavin allows amperometric determination of single nucleotide polymorphism through hydrogen bond-mediated nucleobase recognition in water by using abasic sites as a molecular recognition field.

Much attention has been paid to development of DNA sensing or gene detection techniques. An efficient and highly sensitive method for detecting single-nucleotide polymorphism (SNP) is desired in genome research because SNPs represent genetic variants associated with susceptibility to various common diseases and responses to various drugs. In the area of medical diagnostics, DNA biosensor protocols must provide rapid, sensitive and precise detection of DNA hybridization. Furthermore, it is also highly desirable to construct these DNA biosensors in an array to achieve high throughput assays. Until now, the most commonly used protocols in the area of DNA chip technology have focused on the use of labelling techniques that involve the detection of fluorescent light or electrical signals associated with the hybridization of a probe DNA to the target DNA.[1–4] Although these protocols are very sensitive and rapid, labelling of the DNA usually involves significant cost, time and effort. On the other hand, alternative protocols utilizing non-labeled DNA have also been developed.[5–7] These biosensors detect DNA hybridization by employing optical methods,[5] a quartz crystal microbalance[6] or surface plasmon resonance.[7]

There is considerable interest in electrochemical detection of DNA since it has a good potential for the development of DNA sensors. Two classes of electrochemical methods have been commonly employed to detect DNA hybridization. The first class requires covalent modification of the probe DNA sequences with a redox-active molecule.[8] The second class employs signalling molecules, such as electrochemically active intercalators, that are not required to be covalently bound to the DNA.[9] These detection methods are inherently based on the different properties of duplexes. In these assays, discrimination of fully matched DNAs from mismatched DNAs or SNPs depends on the difference in the duplex stability between a probe strand and a fully matched target strand *vs* a mismatched one or the electronic coupling nature within the base stacks. This is a common principle employed in many other assays used in microarray techniques. It is highly desirable to develop an approach to detecting SNP that does not require DNA labelling and does not depend on hybridization efficiency. Moreover, target base specific SNP detection is also required for simple and rapid SNP typing. To the best of our knowledge, electrochemical detection of SNP which has target base specificity has not been reported until now.

Here, we report a novel electrochemical SNP detection method using an abasic site-containing DNA and a hydrogen bond-forming electrochemically ligand. Recently, we have reported that some hydrogen bond-forming ligands (naphthyridines,[10] pterins,[11], flavins[12a,b] and diaminopyrazines[12c]) can recognize target nucleotides through their hydrogen bonds, using the hydrophobic microenvironments provided by an abasic site-containing DNA duplex. The use of the electrochemical response of hydrogen bond forming-ligands leads to a signal appearing on SNP detection, without introducing reporter molecules into probe or target DNA strands *via* covalent bonding. In this work, our system is further developed for the detection of thymine-related SNP by the use of a biotic electrochemically active compound, lumiflavin (LF), as a hydrogen-bond forming ligand. Binding studies by melting temperature and fluorescence measurements have shown lumiflavin and riboflavin clearly recognize target nucleobases at the abasic site *via* the formation of hydrogen bonds.[12]

In this study, 23-meric model DNA duplexes with a mercaptohexyl linker are utilized for the design of an electrode modified by abasic site-containing DNA. Four kinds of target DNAs are used so as to direct the target nucleotide toward the abasic site. These DNAs are referred to as 23T (T = thymine), 23C (C = cytosine), 23A (A = adenine) and 23G (G = guanine). As shown in Scheme 1, the probe DNA was immobilized on the gold electrode *via* chemisorption according to the following procedure: a 50 μl droplet of a 1 μM probe DNA solution was cast onto well-polished gold disk electrodes (1.6 mm in diameter, BAS). An aqueous buffer solution (pH 7) containing 0.1 M NaCl, 0.01 M sodium cacodylate and 1 mM EDTA was used in all experiments. To minimize surface contamination, the electrodes were kept in the buffer solution and were not allowed to become dry throughout the experiments. Electrochemical quartz crystal microbalance experiments revealed that 18 pmol cm^{-2} of probe DNA was immobilized on the electrode.[13] The electrodes were then washed with 20 ml of the buffer solution. To minimize nonspecific adsorption of probe DNA to a bare spot of the gold electrode surface, a 6-mercapto-1-hexanol (MCH) monolayer was formed by immersing into a 1 mM MCH aqueous solution for 1 h. Then a 50 μl of a solution containing appropriate amounts (0.02–1 μM) of

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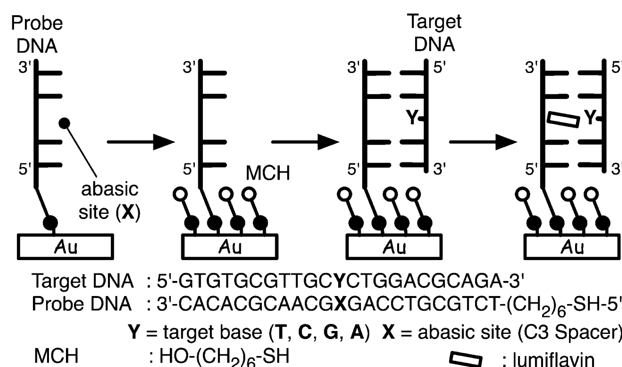
target DNA were cast onto the probe DNA–MCH modified electrode and kept at r.t. for 2 h to allow hybridization. After each exposure the electrodes were rinsed thoroughly using the buffer solution .

The binding of LF toward a target nucleobase was examined prior to the measurement of SNP detection. The DNA modified electrode was immersed into a 50 μM LF solution, followed by rinsing with buffer, and then transferred to a LF-free buffer solution for electrochemical measurements. Fig. 1 shows typical square wave voltammetry (SWV) responses for this electrode treated with different oligonucleotides, such as 23T (target DNA) or 23C, 23G and 23A (non-target DNA). It can be clearly recognized that the electrochemical response is significant in the presence of 23T. The peak current intensity depends on nucleotides opposite the abasic site, and follows the order 23T > 23C > 23A, 23G, while the gold electrode treated with MCH alone has no oligonucleotide moiety and gave no electrochemical response. As previously reported,[12] it is highly likely that LF is incorporated into the abasic site by the binding to nucleotides with selectively for thymine, which results in the increase in the electrochemical response of abasic site-containing DNA duplexes on gold electrode.

The peak potential of the LF which bound to abasic site-containing DNA is −0.370 V (*vs* Ag/AgCl). This value is slightly positive compared with that measured in bulk solution; SWV measurements of an aqueous solution showed a redox potential of −0.415 V. Bard *et al.*[14] reported an electrochemical study on the interaction between electrochemical intercalators and DNA. They showed changes in the electrochemical response of the intercalators and assigned the response as follows: positive shifts in the peak potential were observed in the binding from hydrophobic interactions with the interior region of the DNA duplex, while electrostatic interactions with outer phosphate DNA backbone led to negative shifts. Accordingly, the slight positive shift from −0.415 V to −0.370 V indicates that LF binds to a target nucleobase at the abasic site within a DNA duplex

Next, we further examined the binding behavior of LF by time course SWV measurements. Since SWV measurements were carried out in the absence of LF in the buffer solution, the current intensity decreased along with the measurement time after transferring the electrode into a measurement solution. Fig. 1(b) shows that the normalized current intensity change of LF depends on the time of SWV measurement. The resulting changes in the peak current intensity follow a single exponential decay, indicating that the LF desorbs from the abasic site-containing DNA duplex. The dissociation rate constant calculated by the single exponential decay, is $6.9 \times 10^{-4}\ s^{-1}$ for 23T under the present experimental conditions. For the desorption from other target DNA duplexes (23C, 23G and 23A), LF shows a similar single exponential decay, but the dissociation rate constants are relatively large for these three targets (k_d/s^{-1}: C, 7.6×10^{-4}; A, 1.2×10^{-3}; G, 5.6×10^{-3}). These results indicate that the slower the dissociation rate constant, the stronger the interaction that occurs between LF and target nucleotide in the abasic site. We therefore conclude that LF can recognize thymine base by electrochemical signalling.



Scheme 1 Schematic illustration of the construction of an abasic site-containing DNA duplex modified electrode.

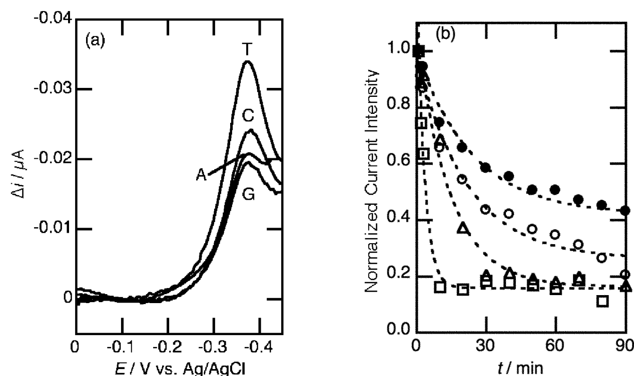


Fig. 1 (a) SWV responses of LF on the electrode modified with an abasic site-containing DNA duplex (**Y** = T, C, G, A) in a buffer solution (pH 7.0). Frequency, 10 Hz; step potential, 5 mV; modulation amplitude, 20 mV. (b) The normalized current intensity–time curve for LF: (filled circle) thymine; (open circle) cytosine; (square) guanine; (triangle) adenine.

Although LF can show a selective electrochemical response to thymine, measurements in a LF-free solution give a time dependent response (Fig. 1(b)) which corresponds to desorption of LF from the abasic site-containing DNA duplexes on the electrode surface, and this feature is not suitable for a routine SNP assay. In order to overcome this disadvantage, a dilute solution of LF, in which the DNA modified electrode was immersed, was used for electrochemical measurements. Fig. 2(a) shows the SWVs recorded on an electrode with abasic site-containing DNA modified electrode before and after hybridization with 1 μM of 23T target DNA. An anodic current due to solubilized LF in the solution is observed both before and after hybridization. Due to the contribution of LF bound to an abasic site DNA duplex, peak current intensity increases after hybridization. The dependence of the peak current ratio (i/i_0) on the varying amount of target DNA is shown in Fig. 2(b), where i_0 and i represent the current intensity before and after hybridization respectively. It can be easily recognized that the value of i/i_0 is highest for 23T target DNA in the concentration range from 0.02 to 1 μM target DNA. This result agree with the SWVs shown in Fig. 1. Therefore, we conclude that the utilization of an abasic-site containing DNA and LF allows amperometric determination of SNP at concentrations below 1 μM of target DNA. Such ratiometric electrochemical assay is indeed sufficient for reliable discrimination of the thymine-related mutation. In addition, since standard PCR protocol can reasonably provide a 0.5 μM DNA sample,[15] the present method would be applicable to PCR amplification products.

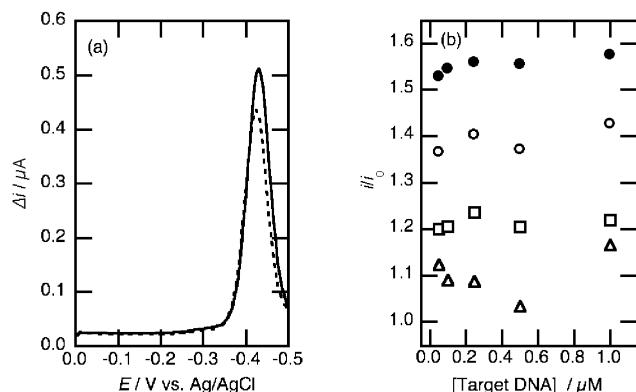


Fig. 2 (a) SWVs of a solution of 25 μM LF in a buffer solution at an abasic site-containing DNA modified electrode (dotted line), after hybridization with target DNA (**Y** = T, solid line). (b) Plot of current intensity ratios of 2.5 μM LF on an abasic site-containing DNA modified gold electrode as a function of target DNA concentration in 10 mM sodium cacodylate buffer solution (pH 7.0) containing 0.1 M NaCl and 1 mM EDTA. i and i_0 denote the current intensities of LF in the 2.5 μM LF before and after target hybridization, respectively: (filled circle) thymine; (open circle) cytosine; (square) guanine; (triangle) adenine.

In summary, we have demonstrated that, in combination with an abasic site-containing DNA duplex modified electrode, LF selectively bound to thymine with a useful electrochemical response, and LF could be successfully utilized for detection of thymine-related mutations. We expect that use of low-molecular-weight ligands will offers a novel approach to a simple, low-cost assay for SNP typing. Furthermore, our method is indeed applicable to the detection of SNPs present in the PCR amplification products. Some studies are in progress for further development of our ligand-based detection method.

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