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Electrochemical detection at low temperature for a specific nucleobase of target nucleic acids by an abasic site-containing DNA binding ligand

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Abstract

Single-nucleotide polymorphism analysis based on fabrication of the abasic site (AP site) in the thiolated DNA duplexes is demonstrated. The AP site allows an electrochemical indicator to bind to target nucleotides accompanied by electrochemical signaling and to detect a specific nucleobase of a target nucleic acid. A mixed self-assembled monolayer of thiolated AP site-containing probe DNA is hybridized with the target DNA so as to direct the AP site toward the target nucleobase. In this way, hydrophobic microenvironments are provided for the electrochemical indicator to recognize the specific nucleobase on the DNA duplex based on the difference in the electrochemical response. The electrochemical response is found to be strengthened at low temperature. The enhanced electrochemical response of riboflavin induced by temperature in an AP site-containing DNA duplex is used to detect thymidine specifically based on an electrochemical approach.

Keywords: Riboflavin; Abasic site; Electrochemical detection; Square wave voltammetry; Biosensor; Single-nucleotide polymorphisms

1. Introduction

The increasing amount of information available on the human genome requires a quick and simple method for the detection of single-nucleotide polymorphisms (SNPs). To date, the conventional SNP detection technologies have been unable to identify all possible SNPs, necessitating further developments in speed and sensitivity at lower cost. We have recently proposed a new strategy for ligand-based fluorescence assay to provide SNP typing with synthetic and/or biotic small ligands possessing hydrogen-bonding groups suitable for nucleotide recognition, in combination with abasic site (AP site)-containing DNAs [1–4]. The loss of a purine or pyrimidine base, resulting in the formation of an AP site, is the most frequent lesion that occurs in DNA. In the course of our study on SNPs typing [1–4], we have used a synthetic oligo-DNA strand containing an AP site to make a hydrophobic space as a recognition field for nucleobase, because a quick, simple and cost-effective method for the routine detection of SNPs is highly desirable [5]. An electrochemical method offers great advantages for point-of-care detection of specific nucleic acid sequences over the existing devices based on optical schemes, because of its potential for being implemented with simple, low-cost, and portable devices. In the last few years, a variety of protocols have been demonstrated to transduce DNA hybridization electrochemically based on long-range electron transfer through well-stacked duplexes

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in which several kinds of DNA intercalators have been used. Redox probe reporters that intercalate into the DNA base stack appear to be a necessary component for detection [6–9].

In contrast to current methods based on hybridization, our approach is based on using AP site-containing thiolated probe DNAs fabricated on the electrode, which allows the use of a small ligand to selectively recognize target nucleotides with electrochemical signaling. The selectivity and high affinity of the ligand–nucleotide binding are improved at low temperature. Here, we describe electrochemical detection of a DNA single base by an AP site-containing DNA binding ligand.

2. Experimental

2.1. Apparatus

Square-wave voltammetry (SWV) was done with an electrochemical voltammetric analyzer, Autolab PGSTAT 100 (Eco Chemie B.V. Utrecht, The Netherlands). A 10 ml electrochemical cell (BAS, Japan) was connected to a temperature controlled water bath. The detection was carried out with gold disk electrodes (1.6 mm in diameter, BAS), a reference electrode (Ag/AgCl, Bioanalytical Systems, West Lafayette, IN), and a platinum wire as the auxiliary electrode at 5, 10, 15, 20 and 25 °C. All measurements were done in 10 mM sodium cacodylate (cacodylic acid sodium salt) buffer solutions (pH 7.0) containing 100 mM NaCl and 1.0 mM EDTA.

2.2. Reagents

Riboflavin was obtained from Aldrich Chemical Co. (Milwaukee, WI) and used as received. 6-Mercapto-1-hexanol (MCH) was obtained from Sigma–Aldrich Co. (USA). All of the oligodeoxynucleotides used in the present study were custom synthesized by Nihon Gene Research Laboratories Inc. (Sendai, Japan). For the synthesis of AP site-containing DNAs, a propylene residue (Spacer phosphoramidite C3, i.e., Spacer C3) was utilized. The probe DNA without an AP-site was also provided for the control experiment. Probe DNA: HS-$(CH_2)_6$-5′-TCTGCGTCCAG**X**GCAACGCACAC-3′ (X = Spacer C3) Target DNA: 3′-AGACGCAGGTC**Y**CGTTGCGTGTG (Y = T, C, G, A) Probe DNA without AP site: HS-$(CH_2)_6$-5′-TCTGCGTCCAG**X**GCAACGCACAC-3′ (X = A).

The concentration of DNA was determined from the molar absorption coefficient at 260 nm according to the literature [10]. Water was deionized ($\geqslant$18.0 MΩ cm specific resistance) with an Elix 5 UV Water Purification System and a Milli-Q Synthesis A10 system (Millipore Corp., Bedford, MA). Other reagents were commercially available analytical grade and were used without further purification. All measurements were done in 10 mM (cacodylic acid sodium salt, $(CH_3)_2AsO_2Na$) buffer solutions (pH 7.0) containing 100 mM NaCl and 1.0 mM EDTA.

2.3. Preparation of duplex-modified gold electrode

A gold electrode was polished successively using 0.1 and 0.05 μm alumina slurries, rinsed with water and then treated electrochemically in 0.05 M H_2SO_4 by cycling the electrode potential 10 times between −0.1 and +1.5 V vs. Ag/AgCl at a scan rate of 0.1 V/s. After that, a 50 μl droplet of a 1 μM thiolated probe DNA solution was cast onto the treated gold electrode (0.02 cm^2 in area). The electrode was kept under saturated vapor pressure overnight. In order to minimize non-specific adsorption of probe DNA, a 6-mercapto-1-hexanol (MCH) monolayer was formed by immersing the electrode into a 1 mM MCH aqueous solution for 1 h. Then, 50 μl of 1 μM target DNA was cast onto the probe DNA-MCH-modified electrode, heated to 50 °C and then slowly cooled (1 h) to 25 °C to to allow hybridization. Finally, the DNA-modified electrode was immersed into a 50 μM riboflavin (RF) buffer solution for 1 h. The electrode was thoroughly rinsed with 20 ml of a buffer solution after each exposure. The

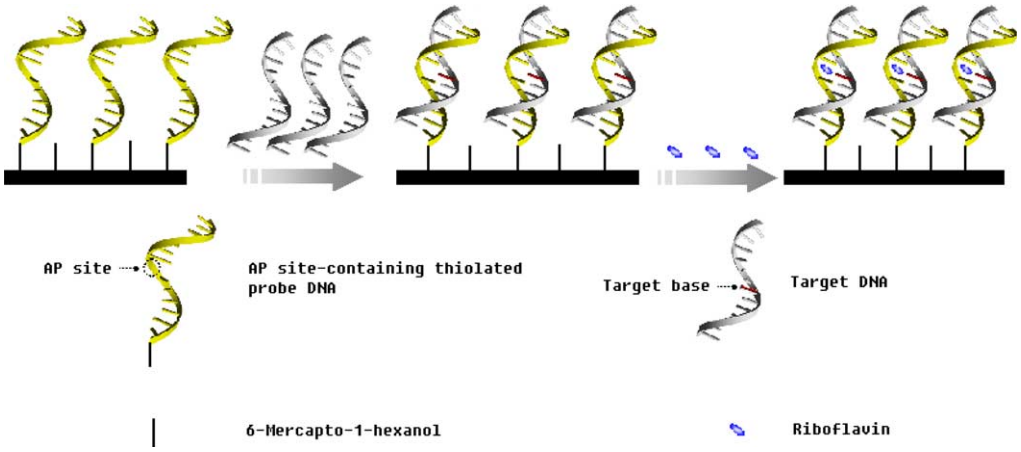

Fig. 1. Schematic illustration of single-nucleotide recognition by riboflavin with AP site-containing DNA duplexes fabricated onto the gold electrode.