Electrochemical investigation of DNA hybridization with ferrocene-labelled peptide nucleic acid on gold electrodes

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Abstract: An electrochemical DNA sensor based on ferrocene-labelled peptide nucleic acid (PNA-Fc) was prepared. The hybridization between PNA-Fc and DNA immobilized on a gold electrode was examined by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). PNA-Fc shows a good electrochemically activity and has a redox potential of 170 mV versus Ag/AgCl electrode after hybridization, representing the characteristic of ferrocene/ferrocenium (Fc/Fc+) transformation. The results illustrate that PNA-Fc can be used as an effective electrochemical DNA probe sensor.

Keywords: peptide nucleic acid; ferrocene; electrochemical sensor

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1 Introduction

Genetic testing is one of the foremost topics in current biochemical research. However, traditional methods of detecting DNA hybridization like gel electrophoresis and membrane blots are too slow and labor intensive. Many different approaches have been proposed for genetic tests [1] including the DNA hybridization sensor, which, in particular, is one of the fastest growing areas in genetic testing technology and has attracted increasing attention of applications in clinical diagnostics, environmental protection, food quality control, and forensic science [2-3]. DNA sensors are small analytical devices. They form a nucleic acid recognition layer immobilized on a physical transducer to recognize by hybridization its complementary target sequence and convert the DNA hybridization event into a useful analytical signal.

Because electrochemical analytical devices are rapid, easy-to-use, inexpensive and miniaturized, electrochemical methods are a better choice than many other methods. One electrochemical method using peptide nucleic acid (PNA) is broadly applied to DNA research. In addition, electrochemistry offers innovative routes for interfacing the nucleic acid recognition system with the signal-generating element and amplifying electrical signals. Thereby, electrochemical methods have drawn great attention in the last few decades. They use small electroactive DNA-intercalating or groove-binding substances as the indicators, which have a much higher affinity for resulting hybrids than a single-stranded probe. The increasing interest in electrochemical methods for hybridization detection arises from the high sensitivity. A current strategy of monitoring DNA hybridization with a complementary strand is based on the change of the electrochemical response of labeling DNA with metal complexes or electroactive compounds. Ferrocene or its derivatives are very good redox-active molecules with excellent redox reversibility and the advantages of being chemically stable, easily functionalized and electrochemically reversible [3-7].

Ferrocenylated oligodeoxynucleotides used as
electrochemical probes of DNA hybridization reactions have been more developed, however, only a few examples of ferrocenylated PNA used as the electrochemical signaling probes have been reported [8-17]. Luo et al. [18] recently focused on the immobilization-free sequence-specific electrochemical detection of DNA using ferrocene-labeled peptide nucleic acid on ITO. PNA represents a very younger promising class of artificial DNA analogues and was described by Nielsen et al. [19] in 1991 for the first time. The replacement of the natural deoxyribose phosphate backbone of DNA by repeating N-(2-aminoethyl)-glycine units with the nucleobases attached through methylenecarbonyl linkers affords a DNA mimic with remarkable properties. In this work, we investigated the electrochemical properties of the hybridization between PNA and ferrocene (Fc) oligomers used as signaling probes and DNA immobilized on the gold surface of the electrode.

2 Experimental details

The ferrocene-labelled peptide nucleic acid (PNA-Fc) were synthesized according to Refs. [20-22]. The gold disk electrode (Au) was polished and electrochemically cleaned. The 5'-thiol tethered ssDNA probe (5'-HS-(CH2)6-AAAAAA-3') was dropped on the cleaned gold electrode surface and kept overnight. Then, the electrode was rinsed with redistilled water for removal of unassembled DNA and placed in a 1 mmol/L 6-mercapto-1-hexnaol solution for 10 min. The modified gold electrode (Au-DNA) was immersed into a PB buffer solution (pH=7.0) containing the desired amount of target PNA oligomers (Lys-TTTTTT or Lys-TTTTTT-Fc) [23]. The hybridization between the immobilized DNA probe on the surface and PNA oligomers in the solution was allowed to proceed at room temperature for 4 h. The gold electrode (Au-DNA-PNA or Au-DNA-PNA-Fc) was then rinsed with a phosphate buffer (PB) to remove non-hybridized target PNA oligomers. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed with a three-electrode (a bare gold electrode as the working electrode with 2 mm diameter, a platinum wire as the counter electrode, and the reference electrode as Ag/AgCl/sat) cell in a PB solution.

It is well known that the probe DNA coverage at the electrode surface is very important to the hybridization. Consequently, the exact value of the probe DNA coverage needs to be measured. The surface coverage of 1.2×10^{-11} mol cm^{-2} DNA was estimated from the Cottrell’s experiment with [Ru(NH3)6]3+ [24] while the surface coverage of [Ru(NH3)6]3+ was calculated as the difference in the chronocoulometric intercepts in the absence and presence of [Ru(NH3)6]3+. The surface coverage of [Ru(NH3)6]3+ was then converted to DNA surface density with \( \Gamma_{DNA} = \Gamma_0 (z/m) \), where \( \Gamma_{DNA} \) is the surface density of DNA, \( \Gamma_0 \) is the surface coverage of [Ru(NH3)6]3+, z is the charge of [Ru(NH3)6]3+, and m is the number of DNA bases.

The voltammetry for a variety of redox molecules at electrodes provides more information about electrode surface [24]. The voltammetry for the redox markers at electrode surfaces is clearly affected by electrostatic interactions with the polyanionic overlayer [25].

3 Results and discussion

The chronocoulometric responses of DNA modified electrodes in the absence and presence of 50 µM [Ru(NH3)6]Cl3 are shown in Fig. 1. The calculated DNA surface density is 1×10^{13}.

Fig. 1 Chronocoulometric responses of DNA modified electrodes in the absence (1) and presence (2) of 50 µM [Ru(NH3)6]Cl3, where \( t \) is time, \( \Omega_t \) is the integrated charge, \( n \) is the number of electrons of a molecule for reduction, \( F \) is the Faraday constant, \( A \) is the electrode area, and \( \Gamma_0 \) is the surface coverage of [Ru(NH3)6]3+.

The reduction of ferricyanide is less reversible at Au-DNA surface than at the Au surface. This is obviously related to the value of oxidation potential \( \Delta E_p \). The increase of \( \Delta E_p \) (from 77 mV to 391 mV) is attributed to repulsive electrostatic interactions impeding the ability of anions to reach the electrode surface (see Fig. 2). The same result was also obtained from the data of DPV and the peak current which decreased from 29 µA to 5 µA (CV curves of DPV are not shown here).
Fig. 3 shows the CV and DPV results of the hybridization between PNA oligomers and surface-immobilized DNA. Fc is a very good redox-active molecule, the peak current for the ferrocene redox couple can be easily seen in cyclic voltammograms (Fig. 3a, $\Delta E_p$ is 38 mV). The redox response of Au-DNA-PNA-Fc is observed (the peak potential $E$ is 0.114 V) from DPV scans for Au-DNA-PNA-Fc. The electrochemical responses of PNA-Fc suggest a promising prospect of applying it to developing DNA electrochemical sensors.

Fig. 4 shows the typical CV curves of the surface-bound Fc molecules after the hybridization of PNA-Fc signaling probe. The CVs suggest quasi-reversible redox reactions seeing that the anodic to cathodic peak current ratio is near linear. The peak current is proportional to the scan rate (Fig. 4b), indicating a surface-bounded PNA-Fc.

**Fig. 2** Voltammetric behaviors of ferricyanide(3-/4-) on an electrode of (1) Au and (2) Au-DNA, where $I$ is the current and $U$ is the voltage between the gold electrode and the reference Ag/AgCl electrode.

**Fig. 3** Curves of (a) CV and (b) baseline-corrected DPV of DNA hybridization with PNA oligomers: (1) Au-DNA-PNA-Fc, and (2) Au-DNA-PNA, where $I$ is the current and $U$ is the voltage between the gold electrode and the reference Ag/AgCl electrode.

**Fig. 4** (a) Cyclic voltammograms of Au-DNA-PNA-Fc measured in PB buffer with a scan rate $v$ of (20, 40, 60, 80, 100, 120, 140, 160, 180 and 200) mV/s, respectively from inside to outside; and (b) the linear relationship between the peak current and the scan rate, where $I$ is the current and $U$ is the voltage between the gold electrode and the reference Ag/AgCl electrode.
4 Conclusion

The modification of DNA immobilized on the surface of a gold electrode by PNA-Fc hybridization was verified by electrochemical measurements. Electrochemical results show Fe-PNA oligomers are very important to electrochemical responses. The particular electrochemical behavior of Fe-PNA suggests promising application to DNA sensors.

References


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