Gene sensor using ferrocenyl oligonucleotide

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An electrochemical gene sensing system has been developed using a redox-active ferrocene-modified oligonucleotide and an oligonucleotide anchored on a gold electrode, both of which formed a sandwich-type ternary complex with the target DNA to give a redox current.

Radioactive isotopes (RI) such as ^{32}P are widely used as labels of DNA because of their high detection sensitivity. However, due to their hazardous nature and short shelf life, the development of an alternative detection system that is convenient and practical without relying on RI is eagerly hoped for. We have proposed electrochemical methods as the alternative[1–3] and proved their potential using a high performance liquid chromatography–electrochemical detector system.[4] Recently, several systems for electrochemical detection of DNA have been reported.[5–11] Here we describe a novel electrochemical technique for gene detection in which a sandwich-type complex from an oligodeoxynucleotide (ODN) immobilized on a gold electrode, the target DNA and a ferrocenyl ODN is formed to give a response (Fig. 1).

All ODNs used in this study were prepared on a fully automated DNA synthesizer (Parmacia, Gene Assembler Plus). The ferrocenyl ODN (**T12Fc**) was synthesized by the coupling of amino-terminated ODN with an activated ester of ferrocenecarboxylic acid according to our previous report.[1,4] A 16-mer ODN (**e16S**) which has five successive phosphorothioate units on its 5′-terminus was synthesized using Beaucage's reagent.[12]

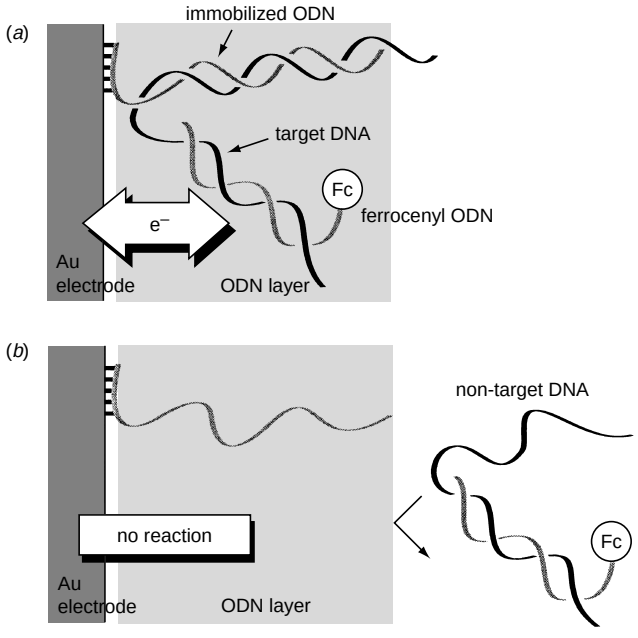
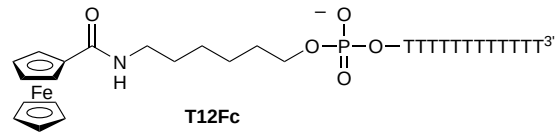


Fig. 1 Schematic illustration of the electrochemical gene sensing system based on the formation of complementary sandwich-type complex. (*a*) Target DNA combines the ferrocenyl ODN with the ODN on electrode. Oxidation current due to the concentrated ferrocenyl units should reflect the concentration of the target. (*b*) Ferrocenyl units are not concentrated onto the electrode using non-target DNA.

e16S 5′TsTsTsTsTsTCTCATACATG3′
e16 5′TTTTTTCTCATACATG3′
t19 5′CATGTATAAAAAAAAAAAA3′
m19 5′CATCTATAAAAAAAAAAAA3′
c19 5′TTCCGCGAAAAAAAAAAAA3′



T12Fc

s = phosphorothioate linkage

The ODN was anchored on the gold electrode *via* chemisorption[13,14] in the following manner: a 10 μl droplet of a 50 μM aqueous solution of **e16S** (containing 100 mM KCl) was cast on well-polished gold disk electrodes (1.6 mm diameter, BAS). The electrodes were then washed with 100 mM KCl solution and stored at 5 °C before use.

Cyclic voltammetric (CV) measurements using a ferrocyanide/ferricyanide redox couple were carried out in order to confirm the ODN modification on the electrode.[13] While the electrode treated with **e16**, which does not have any sulfur atoms, gave the same voltammogram as the bare electrode, the peak current obtained for the **e16S**-modified electrode was significantly suppressed, indicating that the redox couple was excluded from the electrode surface due to electrostatic repulsion between the anionic redox species and the ODN polyanion anchored on the electrode. Heat-treatment of the **e16S**-modified electrode at 80 °C for 30 min in water made no difference to the voltammogram produced. It is likely that the multi-point anchoring of the DNA *via* the phosphorothioate moieties will give stable immobilization of DNA on the gold electrode. This method should be useful for fixation of synthetic DNA onto electrodes.

The electrode modified with **e16S** was immersed in a 50 μM aqueous solution of an equimolar mixture of **T12Fc** and **t19** [**T12Fc**–**t19**, the target containing 100 mM KCl, 5 mM Tris-HCl (pH 8.0)] for 24 h at 5 °C. The electrode was then rinsed with 100 mM KCl at 5 °C. In CV measurements in 100 mM KCl, the modified electrode gave reversible redox waves showing a peak separation of 50 mV. We estimated the amount of the bound probe (**T12Fc**) to be 22–55 pmol cm^{-2} by integration of the charge under the anodic peak. Fig. 2 shows differential pulse voltammograms (DPVs) for the modified electrode. A significant anodic peak due to oxidation of the ferrocene moiety was observed for the electrode treated with the target DNA. On the other hand, only a slight peak was observed for the **e16S**-modified electrode which was treated similarly with a mixture of **T12Fc** and **m19** (**T12Fc**–**m19**, mismatch control) and **T12Fc** and **c19** (**T12Fc**–**c19**, control, data not shown).

The peak potential (E_p) of the DPV was +620 mV (*vs.* Ag/AgCl), which means the redox potential ($E_{1/2}$) of the ferrocenyl unit of **T12Fc** on the electrode was +645 mV [$E_{1/2} = E_p + (\Delta E/2)$]. This value is exceedingly positive compared with that in bulk solution; CV measurements of aqueous **T12Fc** showed a

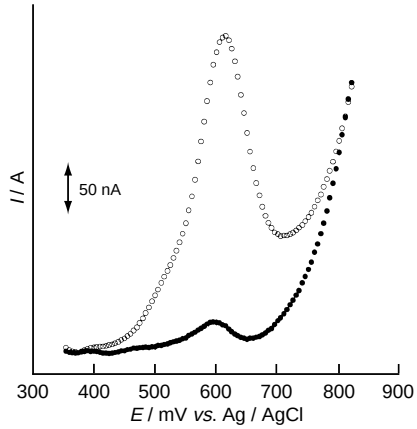


Fig. 2 Differential pulse voltammograms of the ODN modified electrode treated with (○) **T12Fc–t19** (target) and (●) **T12Fc–m19** (mismatch control). The ODN electrode was sensitive only for the target **t19**. Electrodes: Pt plate, Ag/AgCl; pulse period: 200 ms; scan rate: 25 mV s^{-1}; pulse amplitude: 50 mV; pulse width: 50 ms, *T*: 5 °C.

redox potential of *ca.* +430 mV.[1] A similar positive shift was observed in Letsinger's study,[15] in which the ferrocenyl mononucleotide was confined on the electrode surface using sulfur (thiol group)–gold chemisorption.

While the peak for the target sample (**T12Fc–t19**) did not change at all after rinsing the electrode with 100 mM KCl at 5 and 10 °C, similar treatment at 20 °C reduced the peak current appreciably to *ca*. 25%, providing a comparable peak with those obtained for the control samples. On the other hand, the small peaks observed for the control sample (**T12Fc–c19**) and for the mismatch control sample (**T12Fc–m19**) scarcely changed after similar treatment at 20 °C. These results indicate that the binding of the target sample was temperature sensitive. That is, the target was recognized by means of complementary base-pairing with the ODN immobilized on the electrode. On the other hand, the slight current observed in the control and mismatch samples should be ascribed to the non-specific binding of **T12Fc–c19** and –**m19** to the ODN. Thus the detecting device proposed here should be a basis for a simple and highly selective sensing system. Optimization of the measurement conditions such as temperature, salt concentration and/or coexisting non-specific binding blocker (mRNA or sonicated calf thymus DNA) should be made to improve the sensitivity of this system. The electrode's durability also needs to be studied before practical application of this technique to gene diagnosis.

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