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Reliable ferrocenyloligonucleotide-immobilized electrodes and their application to electrochemical DNase I assay

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ABSTRACT

A ferrocenyloligonucleotide (FcODN) having contiguous cytosine bases was immobilized effectively and reproducibly on a gold electrode furnished with a self-assembled monolayer (SAM) having an N-hydroxysuccinimide-activated carboxylic acid. The resulting electrode was used as a sensor chip in DNase I assay. Thus, the current response of the modified electrode decreased upon addition of DNase I, demonstrating that the phosphodiester bonds of FcODN were cleaved. The DNase I activity assessed by Δi defined as $(i_0 - i)/i_0$, where i_0 and i represent the current before and after DNase I treatment, respectively, was found to be reproducible with a standard deviation not greater than 9%. The DNase I can be quantitated in the range of 10^{-5} to 10^{-3} units μL^{-1} with a detection limit of 10^{-5} units μL^{-1} with this sensor chip. The current signal of the FcODN electrode was stable to storage in Biopak water up to 16 days with a 30% signal decrease over this period. DNase I activity in human sera was also determined successfully with this sensor chip.

1. Introduction

Deoxyribonuclease I (DNase I, EC 3.1.21.1), which hydrolyzes phosphodiester bonds of DNA, is implicated in several kinds of disease [1,2]. Abnormality of DNase I results in an increase in DNA concentration in the blood and in turn DNA or DNA–protein complex is accumulated in the blood vessel to cause several autoimmune diseases [3]. For example, glomerular nephritis, arthritis, and anthema are known to be caused by the accumulation of DNA and DNA–protein complex in the blood vessel wall, glomus, or joint and the patient shows low DNase I activity [4–6]. It is also known that DNase I activity in the serum is higher in patients of breast and oral cavity cancers but lower in the serum of lymphoma malignum [7], stomach cancer [8], and myocardial ischemia [12]. It is hence important to monitor DNase I activity for the diagnosis of these syndromes. Several methods are known to assay DNase I activity [4–15]. The single radial enzyme diffusion (SRED) method is known to be highly sensitive [4,5,9,11], but it takes several days to complete a single test. Quicker analysis is possible with newer methods such as the DNase Alert QC System based on a DNA probe with a fluorophore at its terminus and electrochemical ones [11–15].

Recently, we reported on an electrochemical DNase I assay based on the ferrocenyloligonucleotide-immobilized electrode as a sensor chip [15]. The principle of this method is illustrated in Fig. 1. Simple, quick and sensitive as it was, this method suffers poor reproducibility in the immobilization of thiolated ferrocenyloligonucleotides on the gold electrode. To circumvent this problem, DNase I activity was evaluated from the slope of the i_0–i plot against DNase I activity, where i_0 and i refer to the peak currents before and after treatment with DNase I, respectively [15]. Quantitation of the DNase I activity of 10^{-4} to 10^{-2} units μL^{-1} was accomplished by this tweak [15]. Nonetheless, control of the immobilization of a ferrocenyloligonucleotide on the sensor chip remained to be a challenge to further improve the versatility of this method.

In this paper, we tested a new method to immobilize ferrocenyloligonucleotides on the gold electrode through the reaction of contiguous deoxycytidines of a ferrocenyloligonucleotide with N-hydroxysuccinimide (NHS)-activated carboxylic acid on the self-assembled monolayer (SAM) on the gold electrode (Fig. 2). This is the first example of immobilization on gold, though immobilization of native DNA or a synthetic DNA polymer of deoxyguanosine or deoxycytidine on the oxidized glassy carbon electrode was reported previously [16].

2. Experimental

2.1. Materials

Ferrocenylcarbodiimide (FCDI) as a ferrocenylation reagent for single stranded DNA was synthesized according to the route

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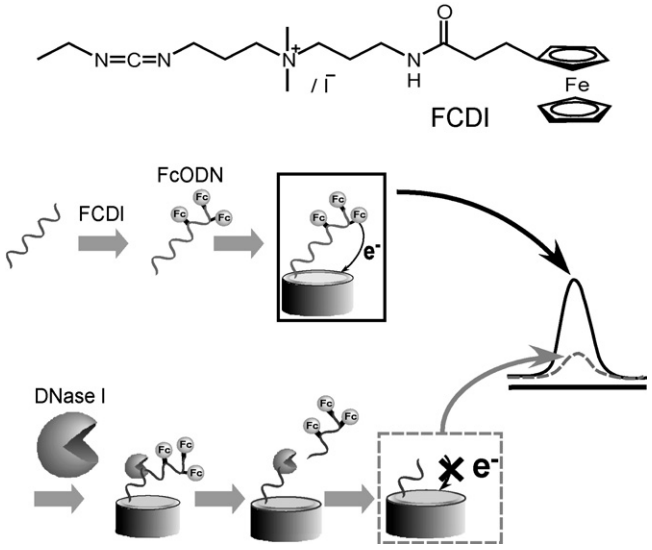

Fig. 1. Chemical structure of FCDI and the principle of electrochemical DNase I assay based on the ferrocenyloligonucleotide-immobilized electrode.

described previously [17]. Oligonucleotide 5′-CCC CCC CCC CCC AAA ACA AAT AAC AAA TAT-3′ (ODN) was custom-synthesized by Genenet (Fukuoka, Japan). Biopak water was purified by a Milli-Q system Gradient A10 coupled with an Elix 3 kit (Millipore, Billeria, MA). DNase I (RNase-free) was purchased from Takara (Shiga, Japan). A 10× DNase I buffer (400 mM tris(hydroxymethyl)aminomethane (Tris)–HCl (pH 7.5), 80 mM $MgCl_2$, and 50 mM dithiothreitol (DTT)) from Takara was used to dilute DNase I. 1-Ethyl-3(3-dimethylaminopropyl)carbodiimide (EDC), N-hydroxysuccinimide (NHS), and 3,3′-dithiodipropionic acid were purchased from Tokyo Chemical Industry (Tokyo, Japan).

2.2. Blood sample preparation

Blood of two of the authors (S.S. and S.T.) was taken at the Center for Student Health of this Institute. After leaving at room temperature for 30 min for coagulation, the blood (4–10 mL) was centrifuged at 3000 rpm for 30 min. The supernatant (serum) was used in DNase I assay after diluting one to one with Biopak water or one containing 100 mM EDTA.

2.3. Synthesis of ferrocenyloligonucleotide (FcODN)

ODN was allowed to react with 100 mM FCDI in 60 μL of 50 mM borate buffer (pH 9.0) containing 30% DMSO at 37 °C overnight and the progress of the reaction was monitored by reversed phase HPLC. Thus, the peak of ODN at 4 min was converted to that at 5 min assignable to the 1:3 adduct (FcODN, 5′-CCC CCC CCC CCC AAA ACA AAT^{Fc} AAC AAA $T^{Fc}AT^{Fc}$, Fc represents the T-modifying ferrocene) of ODN and FCDI. The mass of the product (parent peak) determined by MALDI-TOF MS, 10339.16, was in agreement within error with the theoretical value of 10329.90. FcODN thus obtained was purified finally by reversed phase HPLC.

2.4. Preparation of FcODN-immobilized electrodes

A gold electrode of 2 mm^2 in area (Bioanalytical Systems (BAS), Tokyo, Japan) was polished with 6 and 1 μm of a diamond slurry, and 0.05 μm alumina slurry in this order and sonicated in Biopak water for 5 min (3 times). This electrode was electrochemically polished by scanning 20 cycles from −2.0 to 1.5 V at a scan rate of 100 mV s^{-1} in a 0.5 M H_2SO_4 aqueous solution and sonicated in Milli-Q water for 5 min (3 times). After the electrodes were dried by blowing a nitrogen gas, they were soaked in 300 μL of ethanol containing 1 mM 3,3′-dithiodipropionic acid overnight at 25 °C. After the electrode was washed with Biopak water, it was soaked in 7 mL of an aqueous solution containing 5 mM EDC and 8 mM NHS and kept for 20 min at 25 °C. After the electrode was picked up and held upside down, 50 μL of a proper concentration of FcODN was placed on the electrode at 25 °C for overnight. After washing the electrode with Milli-Q water, 300 μL of 50 mM Tris–HCl, pH 7.4, containing 20 mM NaCl was placed on the electrode to quench any remaining activated carboxyl groups on the electrode for 10 s.

2.5. Apparatus

The HPLC system used in this experiment was composed of the following components: Hitachi C-7300 column oven, L-7450H diode array detector, L-7100 pump and D-7000 interface chromatograph (Hitachi High-Technologies Co., Tokyo, Japan).

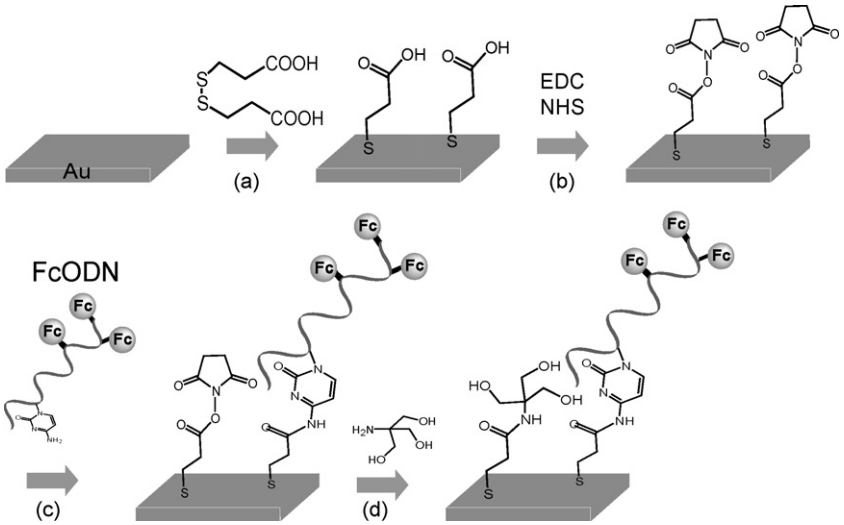

Fig. 2. Preparation of an NHS-activated carboxylic acid on the electrode and immobilization of the ferrocenyloligonucleotide on it. (a) 300 μL of 1 mM 3,3′-dithiodipropionic acid, 25 °C, overnight, (b) 7 mL of 5 mM EDC and 8 mM NHS in water, 25 °C, 20 min, (c) 50 μL of 0.5 μM ferrocenyloligonucleotide, 0.5 M NaCl, 25 °C, 24 h, and (d) 50 mM Tris–HCl (pH 7.4) and 20 mM NaCl, 4 °C, 10 s.