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Electrical Recognition of Label-Free Oligonucleotides upon Streptavidin-Modified Electrode Surfaces

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Abstract For the purpose of developing a direct label-free electrochemical detection system, we have systematically investigated the electrochemical signatures of each step in the preparation procedure, from a bare gold electrode to the hybridization of label-free complementary DNA, for the streptavidin-modified electrode. For the purpose of this investigation, we obtained the following pertinent data; cyclic voltammogram measurements, electrochemical impedance spectra and square wave voltammogram measurements, in $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ solution (which was utilized as the electron transfer redox mediator). The oligonucleotide molecules on the streptavidin-modified electrodes exhibited intrinsic redox activity in the ferrocyanide-mediated electrochemical measurements. Furthermore, the investigation of electrochemical electron transfer, according to the sequence of oligonucleotide molecules, was also undertaken. This work demonstrates that direct label-free oligonucleotide electrical recognition, based on biofunctional streptavidin-modified gold electrodes, could lead to the development of a new biosensor protocol for the expansion of rapid, cost-effective detection systems.

Keywords: direct electrochemical detection, label-free DNA, step-by-step procedure, streptavidin-biotin system

INTRODUCTION

Bio-nanotechnology is rapidly emerging as an important field of scientific study and technological advancement in the 21th century [1-9], particularly in the area of analytical biochip development. The development of biochip technology is considered as to be significantly important for the future development of bio-nanosensors. From both an academic as well as industrial standpoint, the issue of bio-active DNA arrays is important and particularly challenging for the advancement of biochip technology. Generally, protocols for bimolecular detection involve the verification of hybridization with fluorescence or chemiluminescence. While highly effective, such detection methods are often costly, due to the specific reagents and detection equipment required. Although label-free DNA has been developed using optical methods, such as QCM and SPR, these detection methods require a large concentration of DNA. Due to this factor, the development of electrochemical biosensors over the past decade or so has had a significant impact in the areas of diagnostics and genomics [2-5,10-14]. Due to recent break throughs in electrochemical blood-glucose monitoring, there is considerable enthusiasm relating to electrochemical detection and monitoring methods throughout the biotechnology industry.

The electrical signal detection method has recently been adopted for the development of future biosensors, and has proved to be both convenient and economical. However, common electrochemical biosensors, used for the detection of DNA hybridization in redox-active hybridization indicators, bind more strongly to DNA duplexes than to single-stranded (ss) DNA [10]. Therefore, it is necessary to develop clear electrochemical strategies for the hybridization of label-free DNA using soluble mediators. We previously reported electrochemical detection methods for label-free DNA, utilizing hybridization detection, from nucleic acids to protein-bound nucleic acids, using the interaction of streptavidin-biotin with ferri/ferrocyanide solution [6]. Single-base mismatch and negative oligonucleotide targets have also been examined to form the basis of a non-labeled DNA chip in the development of a single nucleotide polymorphism detection system [7]. For electrochemical sensor applications utilizing label-free DNA, it is necessary to research the electron transfer properties of label-free oligonucleotides on biofunctional modified electrodes.

In this report, we present the results of our investigation into the electrochemical signatures during each step of our preparation procedure, from a bare gold electrode to the hybridization of label-free complementary DNA, on the streptavidin-modified electrode; including exami-

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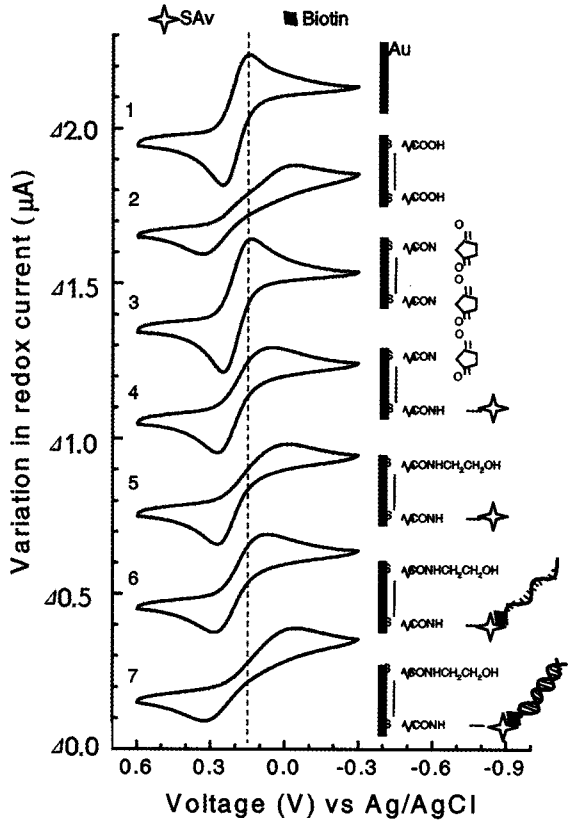

Fig. 1. Cyclic voltammogram series with a scan rate of 200 mV/s, obtained in 0.1 M KCl solution containing 5 mM $Fe_3(CN)_6$, using a bare gold electrode (1), after treatment of a thiol-modified electrode (2), after treatment of a carboxylic group-treated electrode (3), after treatment of a streptavidin modified electrode (4), after treatment of a amino group treated electrode (5), after immobilization of biotinylated ssDNA (6), and after hybridization of complementary label-free DNA (7).

nation of the charge transport properties, using building blocks. In order to generate bio-functional surfaces, the streptavidin-biotin system, which has become a universal molecular system in biological science, is frequently used due to its high affinity constant and stability. In most cases, the streptavidin is bound as a monolayer to the transducer surface. Basically, as any biotinylated molecule may be bound irreversibly, the streptavidin-functionalized surfaces may be used for the detection of a variety of analytical target molecules.

MATERIALS AND METHODS

A bare gold electrode, 200 μm in diameter, was fabricated on a glass surface, using the sputtering method and photolithography technique. For the electrochemical measurements, the gold electrode was potential scanned 10 times in a 10 mM H_2SO_4 solution, at a scan rate of 100 mV/s, to remove the material impurities, within the range 0 to +1.8 V. Additionally, the bare gold electrode was treated, utilizing an electrochemical method, to fabricate a clean surface, by repeated potential scanning within the range 0 to +1.0 V, in a 5 mM $K_3Fe(CN)$ solution containing 0.1 M KCl, at a scan rate of 200 mV/s (step 1). A flat bare gold surface is an important factor in the reproducibility and stability of electrochemical measurements. The AFM (atomic force microscopy) image has previously been reported by us [5]. The bare gold electrode, thus cleansed, was then soaked in an aqueous solution of 1 mM 3,3′-dithiodipropionic acid, for 40 min at room temperature, in order to apply a self-assembled thiol monolayer (step 2). After washing with PBS (phosphate buffered saline) buffer solution, the electrode was dropped into a carboxylic acid solution, containing 1 mM N-hydroxysuccinimide (NHS) and 1 mM N-Ethyl-N′-(3-(dimethyl) aminopropyl) carbodiimide hydrochloride (EDC), for 40 min in a high humidity environment, to prevent vaporization during treatment of the electrode (step 3). The modified gold surface formed in step 2 was repeatedly rinsed with water, and then dropped into an aqueous PBS buffer solution containing streptavidin (10 μg/mL), for 1 h, in a high humidity and darkened environment (step 4). The streptavidin-modified electrode was then dropped into amino ethanol (1 mM, pH 8.4) solution for 30 min to deactivate any of the unreacted carboxylic acid (step 5). To immobilize the biotinylated ssDNA probe, using the streptavidin-biotin interaction, the surface was then dropped into ssDNA (50 μM), for 1 h at room temperature (step 6). Finally, the hybridization of complementary label-free DNA was carried out by applying an electric field of 500 mV for 30 sec (step 7). Due to its anionic characteristics, the DNA probe can be hybridized with the DNA target onto the electrode through the application of positive biases. The schematic representations for these steps are illustrated in the inset of Fig. 1.

After the above preparation steps, cyclic voltammetry (CV), square wave voltammetry (SWV) and electrochemical impedance spectra were obtained in 5 mM K_3Fe (CN) solution containing 0.1 M KCl. All electrochemical experiments were carried out in a conventional three-electrode system. A Ag/AgCl electrode was utilized as the reference electrode, with a platinum wire (1 mm) as the counter electrode. Electrochemical experiments were performed using a CHI1030 multi-potentiostat (Austin, TX, USA) at room temperature. The CV recordings were conducted in the potential range -0.25 to 0.6 V, with a scan rate of 100 mV/s. SWV was also utilized, with pulse amplitude and pulse width settings of 50 mV and 50 ms, respectively. Impedance spectra were recorded in the reduced fraction, at electrode potentials (E) within the frequency range 0.01 to 20,000 Hz, with an amplitude of 10 mV. Before each frequency sweep, the electrode was pre-polarized at E for 5 min. Before and after each impedance measurement run, voltammograms were recorded within the potential range -0.15 to 0.55 V to monitor the stability during each step. Streptavidin (Sigma Chemicals Co., St. Louis, MO, USA) was diluted in 250 mM NaCl/50 mM sodium phosphate (pH 7.2), to a final concentration of 10 μg/mL. All DNA synthesis

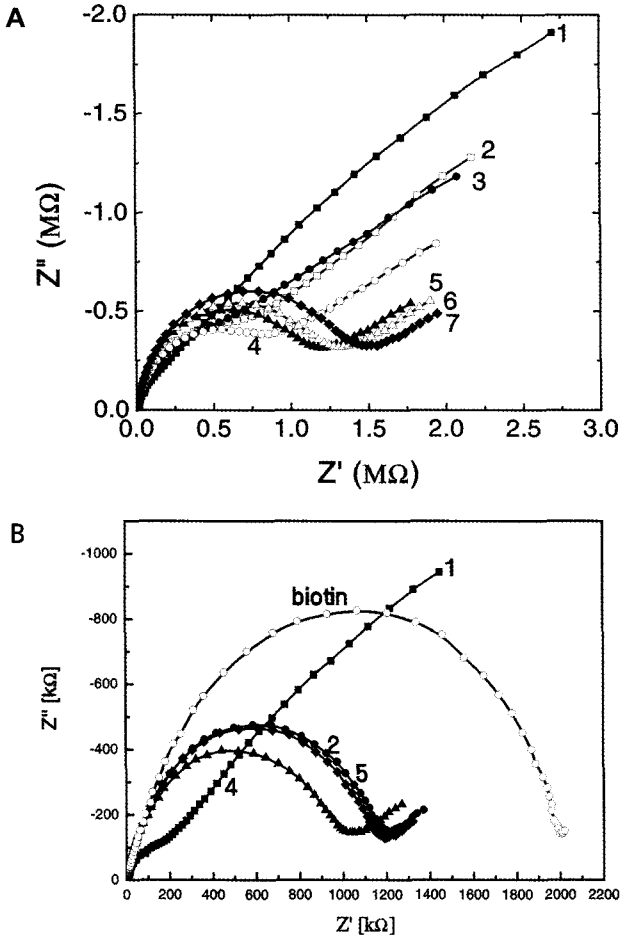

Fig. 2. (A) The faradic impedance spectra from each step. The numbers of each step correspond to those displayed in Fig. 1, with the results similarly corresponding to those of the CV. In steps 1 and 3, a practically straight line is displayed, illustrating mass transport, as in a diffusive system. However, in steps 2, 4, 5, 6, and 7, a semicircle diameter was obtained, indicative of a charge transport type mechanism, as in a kinetic system. (B) The impedance of only biotin (without DNA) as the probing material.

reagents and the biotin were obtained from Nishimbo Co. (Kyoto, Japan). Various kinds of DNA were suspended in TE buffer solution, to a concentration of 50 μM. Four types of biotinylated 21-mer ssDNA sequence were used in probes 1, 2, 3, and 4, respectively. The sequences of each probe were as follows; 5′-biotin-GAGGAGTTGGG GGAGCACATT-3′ (probe 1), 5′-biotin-GAGGAGTTGG GGGAGCACATT-3′ (probe 2), 5′-biotin-GAGGAGGTG GGGGAGCAGGAG-3′ (probe 3), and 5′-biotin-AAGGC CAGCACGTGAAGAAGA-3′ (probe 4). The complementary target DNA of probe I was 3′-AATGTGCTCCCCC AACTCCTC-5′.

RESULTS AND DISCUSSION

We investigated the electrochemical properties in a methodical, step by step production in order to ascertain the electric recognition characteristics of label-free oligonucleotides on the streptavidin-modified electrode surfaces. Fig. 1 illustrates the series of cyclic voltammograms (CV), from bare gold to hybridization, which were obtained using complementary DNA, with 200 mV/s scan rates, in 5 mM $K_3Fe(CN)_6$ solution containing 0.1 M KCl. The electrode potential at each step was shifted according to the reduction peak position of the bare gold electrode that the mean relative standard deviation is 10%. Also, the redox reaction in all steps was clearly demonstrated by the peaks obtained from both the oxidation and the reduction curves. Regarding the bare gold in step 1, our data show the peak-to-peak separation (ΔE_p) and the oxidation peak current were enhanced at 100 mV and 0.252 μA, respectively. The peak separation ΔE_p observed in step 2, with treated SH groups, was increased to 175 mV, but the oxidation peak current was decreased to as low as 0.163 μA below that of bare gold. This resulted in a small Faradic insulating-current layer on the SH group modified bare gold.

After the EDC and NHS treatments (step 3), the ΔE_p and oxidation peak current were increased to those of bare gold. It is suggested a charge transfer occurred due to tunneling of cationic NH^+ through the activated surface. After the modification of streptavidin in step 4, the electric signal was reduced to 0.177 μA and the peak separation, ΔE_p, increased to 260 mV. Therefore, this indicates that the charge transport was actually interrupted by the streptavidin layer. We have previously reported the AFM image of streptavidin, which was shown to be closely packed on the gold surface [5]. Nevertheless, the explicit redox peaks were indicated to occur through charge transport, *via* diffusion between the streptavidin molecules.

When the amino groups on the streptavidin-modified electrode were treated (step 5), the decreased in the redox peak current signal was likely due to the effect of deactivated amino groups causing the carboxylic groups to lose cohesion with the streptavidin. After immobilization of the biotinylated single-strand (ss) DNA probe onto the streptavidin-modified electrode (step 6), the redox current increased. The oxidation peak current increased from 0.159 to 0.183 μA and the peak separation ΔE_p decreased from 0.341 to 0.240 V. This indicates that much signal transduction, through the immobilized ssDNA molecules, is exposed due to the $[Fe(CN)_6]^{3-/4-}$ electrophore. After hybridization with complementary DNA (step 7), the redox current was reduced, showing a larger ΔE_p (0.363 V). The large peak separation was indicative of a slowing of the electron transfer rate, as the negative charge of hybridized dsDNA, with complementary DNA, was prevented by electron transfer in the $[Fe(CN)_6]^{3-/4-}$ solution.

Fig. 2A illustrates the faradic impedance spectra for each step. The numbers for each step correspond to those displayed in Fig. 1, with the results also corresponding with those of the CV. In steps 1 and 3, a practically straight line was obtained, illustrating mass transport, as in a diffusive system. However, in steps 2, 4, 5, 6,

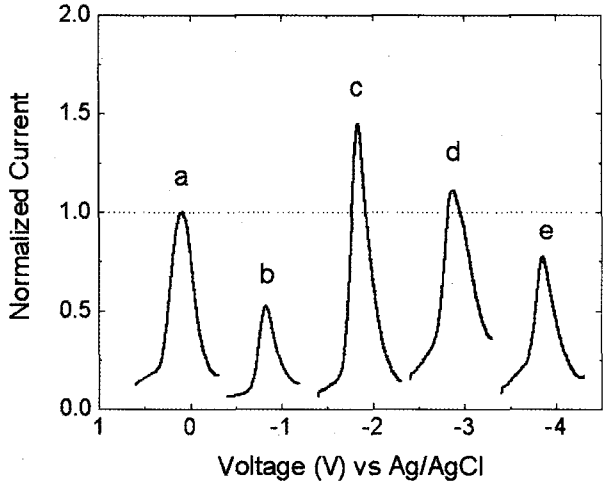

Fig. 3. Square wave voltammograms of (a) step 6 in Fig. 1, (b) biotin only, without DNA on the streptavidin modified electrode, and (c), (d), and (e) other types of biotinylated DNA sequence. The data were normalized to the peak current of step 5, and shifted based on equal values.

and 7, a semicircle diameter was obtained, indicative of a charge transport type mechanism, as in a kinetic system. From each step in the modification of streptavidin in the electrode preparation, the increase in the electron-transfer resistance was attributed to the hydrophobic insulation of the electrode perturbing the interfacial electron transfer. However, in the impedance spectra of biotin only, without DNA, as shown in the Fig. 2B, the semicircle diameter was more pronounced than with biotinylated DNA, implying the binding of biotin only results in a pronounced increase in the interfacial electron-transfer resistance. Immobilization of ssDNA molecule on streptavidin-modified gold electrode causes an electrostatic repulsion between the negatively charged DNA molecules. The electrostatic repulsion enlarges the intermolecular void between streptavidin molecules through which permeation of $Fe(CN)_6^{3-/4-}$ ions is more easily allowed [16]. Therefore, it is suggested from the electrochemical measurement of biotinylated oligonucletide molecules, in ferricyanide solution, is only slightly helpful to electron transfer.

In order to further understand the increasing redox current of biotinylated ssDNA on the streptavidin-modified gold electrode (in step 5, probe 1), the square wave voltammogram (SWV) signals were also investigated under other conditions for the probing molecules (probe 1, only biotin, probe 2, probe 3, probe 4) as illustrated in Figs. 3a, b, c, d, and e. The SWV signal, rather than that of the CV, can provide a larger electrochemical change because the former can suppress the capacitive current in the latter, which is due to the charging effects within the protein layer. The SWV current when probing molecules under the other conditions was normalized by the redox peak current of the biotinylated ssDNA with probe 1 (Fig. 3a). Fig. 3b shows the redox signal after interaction with biotin only, in the absence of DNA molecules; the redox current was significantly decreased (approximately 50%). Conversely, the signal after the probe had been biotinylated with DNA increased with the different steps, as illustrated in Figs. 3c and d. In the case of probe 4; however, the redox current was slightly decreased, as shown in Fig. 3e. It can be suggested that the redox peak current after immobilization of biotinylated ssDNA on modified streptavidin electrode was contributed upon the charge of ssDNA sequence. This matter requires further exploration which the affect may well be related to electrochemical signal.

In conclusion, we have systematically investigated the electrochemical signatures of each step in the preparation procedure, from bare gold electrode to the hybridization of label-free complementary DNA, on the streptavidin-modified electrode, using CV, electrochemical impedance spectra and SWV measurements. A redox mediator of the electron transfer, a $Fe(CN)_6^{3-/4-}$ solution, was used. The oligonucleotide molecules on the streptavidin- modified electrodes exhibited an intrinsic redox activity in the ferrocyanide-mediated electrochemical measurements. Furthermore, investigation into the electrochemical charge transport, according to sequence of oligonucleotide molecules, was also investigated. This work demonstrates that direct label-free electrochemical detection, based on the use of a streptavidin-modified gold electrode, using ferricyanide redox species, provides possibilities that could lead to the development of a new biosensor protocol for the expansion of rapid and cost-effective detection systems.

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