An Amphiphilic Polymer- and Carbon Nanotube-Modified Indium Tin Oxide Electrode for Sensitive Electrochemical DNA Detection with Low Nonspecific Binding

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Abstract
We herein report an amphiphilic polymer-, carboxylated multiwalled carbon nanotube (CNT)-, silane polymer-, and streptavidin-modified indium tin oxide (ITO) electrode that allows low nonspecific binding and efficient immobilization of DNA, along with good electrocatalytic activities and low background-current levels. The low nonspecific binding results from the well-covering of the CNT and ITO surface with the amphiphilic polymer and silane polymer, as well as the poly(ethylene glycol) groups of the polymers. The streptavidin for DNA immobilization is covalently attached to the carboxylic acid groups of the amphiphilic polymer and CNT. A low surface coverage of CNT on the ITO electrode provides the good electrocatalytic activities and low background-current levels. The fabricated electrode enables us to achieve a detection limit of 100 pM in DNA detection.

Keywords: Carbon nanotubes, Amphiphilic polymer, Nonspecific binding, Indium tin oxide, DNA sensors, Nanotubes, Biosensors

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In recent years, carbon nanotubes (CNTs) have been popular electrode materials due to their high electrocatalytic activities and high electrical conductivities [1–4]. In particular, in electrochemical (bio)sensors, CNT-modified electrodes have been widely used as sensing electrodes to achieve high electrochemical signals at low overpotentials [4–8]. Regarding DNA detection, however, the use of CNT-modified electrodes is quite limited given the high nonspecific binding of single-stranded DNA to the CNT surface [9,10]. Although this nonspecific binding allows immobilization of single-stranded DNA on the CNT surface and aids in CNT water-dispersion [9,10], the adsorbed portions of the DNA are not readily hybridizable. Moreover, the additional nonspecific binding of DNA to the DNA-immobilized CNT surface during the biosensing process significantly increases background levels of the DNA sensors, resulting in poor sensitivities. Therefore, CNT-modified electrodes that can provide efficient immobilization and low nonspecific binding of DNA are essential for sensitive DNA detection.

We have recently shown that an amphiphilic polymer consisting of two parts (hydrophobic dodecyl group, hydrophilic poly(ethylene glycol) (PEG) group) makes CNTs water-dispersible and resistant to nonspecific protein binding [11]. Furthermore, an amphiphilic polymer consisting of three parts (dodecyl group, PEG group, amine-reactive functional group) allowed facile polymer adsorption on polystyrene plastic surface, low nonspecific binding of proteins, and facile immobilization of proteins [12]. Importantly, these polymers can be readily coated in an aqueous environment on any hydrophobic surface.

Generally, immobilization of biomolecules is achieved by covalent [13–15] or biospecific binding [15–18]. Among the numerous immobilization methods, the biospecific binding between biotin and avidin (or streptavidin) is most commonly employed. For DNA immobilization, biotinylated DNA is bound to avidin- or streptavidin-modified surfaces [18–20]. Interestingly, avidin readily adsorbs onto hydrophobic surfaces due to its carbohydrate moiety, but in many cases the avidin-adsorbed surfaces allow very low nonspecific binding of proteins [21,22]. However, the high isoelectric point of avidin ($pI = 10$–10.5) [17,23] causes high nonspecific binding of negatively charged DNA under neutral pH. Conversely, streptavidin-modified surfaces allows low nonspecific binding of DNA [18], while immobilization of streptavidin on solid surfaces through nonspecific binding only is not effective.

In this communication, an amphiphilic polymer-, carboxylated multiwalled CNT (CNT)-, silane polymer-, and streptavidin-modified indium tin oxide (ITO) electrode is reported. The modified electrode allows low nonspecific

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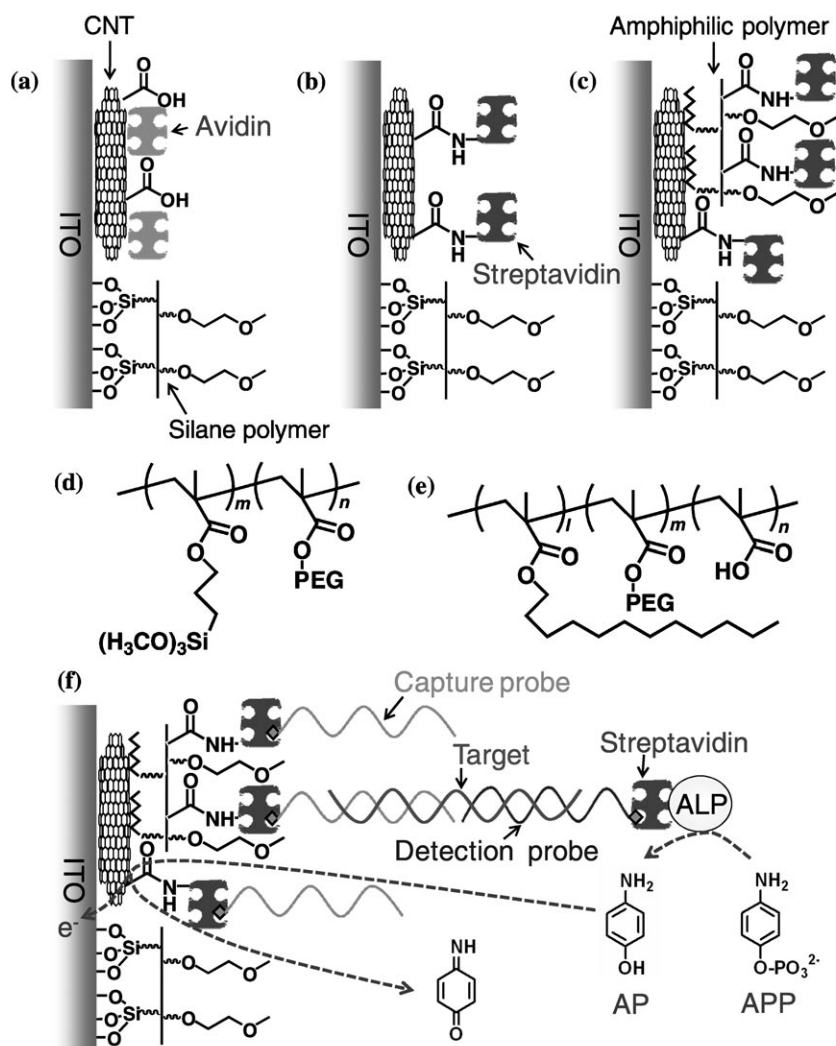

Fig. 1. Schematic diagrams of (a) avidin-modified; (b) streptavidin-modified; (c) amphiphilic polymer- and streptavidin-modified surface. Chemical structure of (d) silane polymer; (e) amphiphilic polymer. (f) Schematic sensing scheme of the electrochemical DNA sensor.

binding and efficient immobilization of DNA, along with good electrocatalytic activities and low background-current levels. Nonspecific binding to the modified electrode was compared with that to avidin- or streptavidin-modified electrodes prepared without using the amphiphilic polymer. The fabricated electrode was then applied to detect target DNA.

The CNT-modified electrodes were prepared by forming a low surface coverage of CNT on ITO electrodes (Figure 1a–1c), allowing both good electrocatalytic activities and low background-current levels [21]. The CNT was physically adsorbed onto the ITO electrodes in an aqueous solution of CNT; the surface coverage of the CNT (the relative fraction of the surface occupied by the CNTs) was ca. 0.03 [21]. After CNT adsorption, the unmodified, vacant regions of the ITO electrodes were covered with a dense polymeric monolayer of silane polymer (Figure 1d) [21]. The silane group of the polymer was used for binding to ITO electrodes, while the PEG group was used to repel nonspecific biomolecular binding [21,24].

To immobilize biotinylated capture-probe DNA, the electrodes should be modified with avidin or streptavidin. Three avidin- or streptavidin-modified surfaces (Figure 1a–c) were compared in terms of relative nonspecific binding of DNA. In Figure 1a, avidin was nonspecifically adsorbed onto a hydrophobic CNT surface [21,22]. In Figure 1b, streptavidin was covalently attached to a carboxylic acid group of CNT [25]. In Figure 1c, the amphiphilic polymer (Figure 1e) was adsorbed onto the CNT surface [11], and streptavidin was then covalently attached to the carboxylic acid group of the polymer and CNT [25]. The dodecyl group of the polymer was used to adsorb onto the hydrophobic CNT surface, while the PEG group was used to repel nonspecific biomolecular binding [11]. After the avidin- or streptavidin-modified electrodes were immobilized with biotinylated capture probe-DNA, the electrodes were incubated in a hybridi-

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zation buffer (HB) not containing target DNA and then treated with biotinylated detection-probe DNA and ALP (alkaline phosphatase)-conjugated streptavidin (Figure 1f). If nonspecific binding of the detection probe and/or ALP-conjugated streptavidin is high, many electroactive *p*-aminophenol (AP) molecules are generated from *p*-aminophenylphosphate (APP) by enzymatic reaction of ALP (Figure 1e). Figure 2 shows cyclic voltammograms obtained with the three different surfaces in an APP-containing solution. The anodic peaks near 0.2 V were due to AP oxidation, while the anodic currents above 0.4 V to APP oxidation [21]. When the CNT is not present on the ITO electrode, the anodic peak for AP oxidation appears at a potential more positive than 0.4 V, which makes it difficult to obtain a high current of AP oxidation without interference of APP oxidation [21]. The good electrocatalytic activity of the CNT-modified electrode allows to oxidize AP at low overpotentials and allows to achieve a high current without the interference. In the case of the avidin-modified surface (curve i of Figure 2) and streptavidin-modified surface (curve ii of Figure 2), the anodic currents related to AP oxidation were very high. However, in the case of the amphiphilic polymer- and streptavidin-modified surface (curve iii of Figure 2), the anodic currents related to AP oxidation were much smaller and slightly higher than those in the absence of nonspecific binding, indicating that nonspecific binding of the detection probe and/or ALP-conjugated streptavidin to the amphiphilic polymer- and streptavidin-modified surface was much smaller. The low isoelectric point of streptavidin ($pI=5$) [17,23] allows low electrostatic binding between streptavidin and DNA in neutral solutions; the well covering of CNT and ITO surfaces with the amphiphilic polymer and silane polymer, as well as the PEG group of the polymers, renders the surface highly resistant to nonspecific bimolecular binding [11,21]. The high nonspecific binding to the avidin-modified surface seems to be due to the high isoelectric point of avidin, which causes electrostatic binding between positively charged avidin and negatively charged DNA. The high nonspecific binding to the streptavidin-modified surface seems to be due to DNA and/or ALP-conjugated streptavidin binding to the unmodified CNT surface.

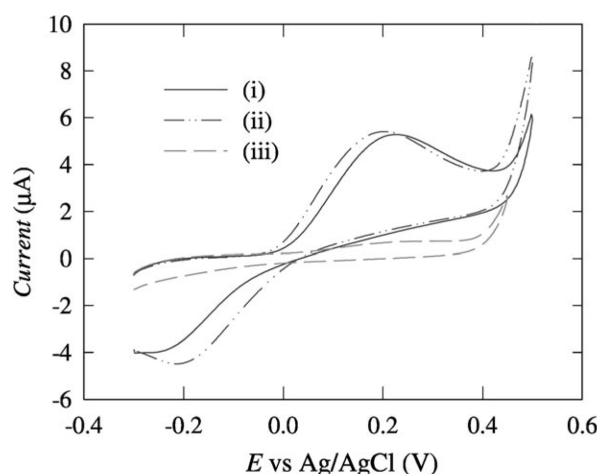

Fig. 2. Cyclic voltammograms obtained with the DNA sensor using (i) avidin-modified surface of Fig. 1a; (ii) streptavidin-modified surface of Fig. 1b; (iii) amphiphilic polymer- and streptavidin-modified surface of Fig. 1c at a DNA concentration of zero. Cyclic voltammograms were obtained at a scan rate of 20 mV/s after incubation for 10 min in a tris buffer containing 2.0 mM APP.

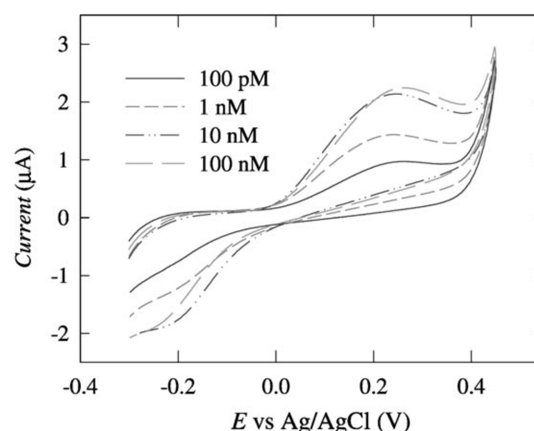

Fig. 3. Cyclic voltammograms obtained with the DNA sensor of Fig. 1e in different DNA concentrations. Cyclic voltammograms were obtained at a scan rate of 20 mV/s after incubation for 10 min in a tris buffer containing 2.0 mM APP.

Figure 3 shows the dependence of cyclic voltammogram on target-DNA concentration. The anodic peak current at a DNA concentration of 100 pM (1.12 ± 0.26 μA (mean ± standard deviation)) was higher than that at a DNA concentration of zero (0.75 ± 0.03 μA) (curve iii of Figure 2), showing that the amphiphilic polymer- and streptavidin-modified surface allows efficient immobilization of the biotinylated capture-probe DNA and that the immobilized DNA is hybridizable. The anodic peak current increased with increasing DNA concentration. Figure 4 shows a calibration plot of the DNA detection. The calculated detection limit for DNA was ca. 100 pM.

In summary, a sensitive electrochemical DNA sensor using an amphiphilic polymer-, CNT-, silane polymer, and streptavidin-modified ITO electrode has been developed. The well-covering of the CNT and ITO surface with the polymers, the PEG group of the polymers, and the low isoelectric point of streptavidin ($pI=5$) make the electrode surface highly resistant to nonspecific DNA binding.

Experimental

Ethylenediaminetetraacetic acid (EDTA), *N*-(3-dimethylaminopropy)-*N*-ethylcarbodiimide hydrochloride (EDC), and *N*-hydroxysuccinimide (NHS) were obtained from Sigma-Aldrich. The APP was obtained from Biosynth (Staad, Switzerland). The CNT was prepared as described previously [21]. Avidin, streptavidin, and ALP-conjugated

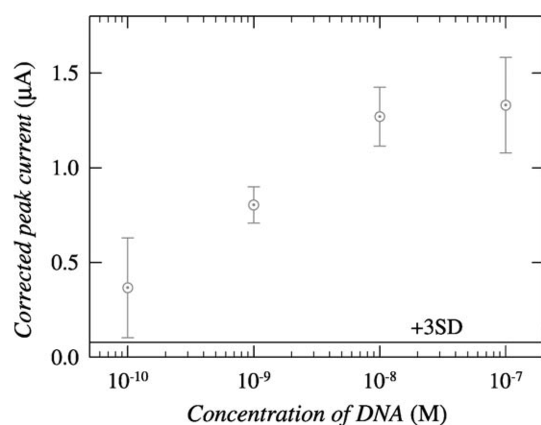

Fig. 4. Calibration plot of the DNA sensor of Fig. 1e. All data were subtracted by the mean peak current at a DNA concentration of zero. The dashed line corresponds to three times the standard deviation (*SD*) of the peak current at a DNA concentration of zero. The error bars represent the *SD* of at least three measurements.

streptavidin were obtained from Sigma-Aldrich. Poly(-TMSMA-*r*-mPEGMA) were synthesized using (trimetoxysilyl)propyl methacrylate and poly(ethylene glycol) methacrylate, as described previously [21]. Poly(DMA-*r*-mPEGMA-*r*-MA) was synthesized using dodecyl methacrylate, poly(ethylene glycol) methyl ether methacrylate, and methacrylic acid, as described previously [11,12]. All DNA was obtained from Genotech (Daejeon, Korea). The DNA sensor was designed for the detection of the encoding residue 1038 of exon 11 of the *BRCA1* gene. The DNA had the following sequences: biotinylated capture-probe DNA, biotin-C_6-5′-AAA GAA GCC AGC TCA A-3′; complementary target DNA, 5′-CTT CAT TAA TAT TGC TTG AGC TGG CTT CTT T-3′; biotinylated detection-probe DNA, 5′-GCA ATA TTA ATG AAG-TTT TT-biotin-3′. All reagents for buffer solutions were supplied by Sigma-Aldrich.

The PBS buffer consisted of 0.01 M phosphate, 0.138 M NaCl, and 0.0027 M KCl (pH 7.4). The PBSB buffer contained all of the ingredients of PBS, with an additional 1% (w/v) albumin-bovine serum (pH 7.4). The rinsing buffer (RB) consisted of 50 mM tromethamine, 40 mM HCl, and 0.5 M NaCl. The binding buffer (BB) contained 50 mM tromethamine, 40 mM HCl, 0.3 M NaCl, 0.5% (v/v) Tween 20, and 1% (w/v) albumin-bovine serum. The HB (pH 7.4) contained 20 mM tromethamine, 17.5 mM EDTA, 150 mM NaCl, and 0.05% (v/v) Tween 20. The tris buffer for electrochemical experiments contained 50 mM tromethamine, 10 mM KCl, 1.0 g/L $MgCl_2$, and 7.0 mM HCl (pH 9.0).

Modification of the ITO electrode with CNT and silane polymer was performed according to our previous report [21]. For adsorption of the amphiphilic polymer (Figure 1c), the modified ITO electrode was immersed in an aqueous solution of amphiphilic polymer (10 mg/mL) at room temperature for 2 h, washed with water, and dried at 50 °C [11]. For immobilization of avidin (Figure 1a), the modified ITO electrode was immersed in a PBS solution containing 100 μg/mL avidin for 6 h [21]. For covalent attachment of streptavidin to the carboxylic groups (Figure 1b and 1c), the modified electrode was immersed in an aqueous solution containing 50 mM EDC and 25 mM NHS at room temperature for 2 h, and then immersed in a PBS solution containing 0.1 mg/mL streptavidin at 25 °C for 2 h [25]. Afterward, the avidin- or streptavidin-modified electrode was washed with PBS and immersed in BB for 30 min. After washing the electrode with PBS, the electrodes were immersed in PBS containing 1.0 μM biotinylated capture-probe DNA, followed by washing with RB. The HB containing different concentrations of target DNA was loaded onto the electrode and maintained at room temperature for 2 h for hybridization. Next, the electrode was washed with RB, and the HB containing 1.0 μM biotinylated detection-probe DNA was loaded on the electrode and maintained at room temperature for 2 h. After washing with RB, the electrode was dipped in BB, containing 10 μg/mL ALP-conjugated streptavidin, followed by washing with RB.

The electrochemical experiment was performed using a CHI 617B or CHI 708C (CH Instruments, Austin, TX, USA). The electrochemical cell consisted of a modified ITO working electrode, a Pt counter electrode, and an Ag/AgCl reference electrode.

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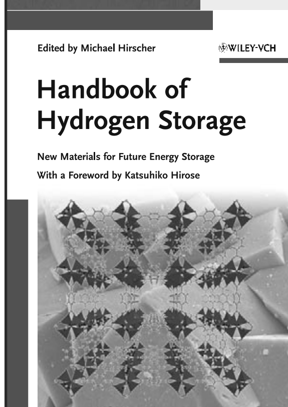

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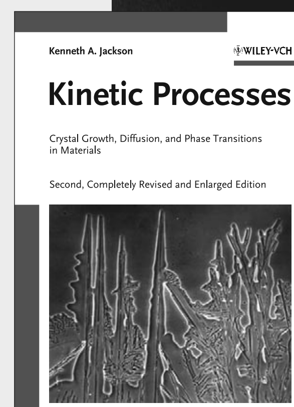

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