Notes

Preparation of Layer-by-Layer Thin Films Composed of DNA and Ferrocene-Bearing Poly(amine)s and Their Redox Properties

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Introduction

Layer-by-layer (LBL) thin films composed of biopolymers have received considerable attention because of the possible applications to drug delivery, bioreactors, and biosensors.[1] Among biopolymers, proteins have most extensively been used for developing biologically active LBL films, in which, for example, catalytic activity of enzymes and/or binding activity of antibodies and other proteins are preserved. The protein LBL films have been deposited on the surface of an electrode to construct electrochemical biosensors.[2] DNA has also been used for preparing LBL thin films by taking advantage of the fact that DNA contains negative charges originating from phosphate residues along the polynucleotide chain. The construction of DNA-containing LBL films and evaluation of the structure was first reported by Sukhorukov and co-workers.[3] Recently, the binding of dyes to DNA films and their controlled release and the stimuli-sensitive and enzymatic degradation of LBL DNA films have been extensively studied.[4] In addition, DNA-containing LBL films have been used for constructing electrochemical DNA sensors that can detect DNA damage and hybridization, using redox-active polycations containing $[Ru(bpy)_2Cl]^{2+}$ (bpy = 2,2′-bipyridine) and naphthoquinone side chains.[5] Poly(4-vinylpyridine) derivative bearing $[Os(bpy)_2Cl]^{2+}$ has also been used for preparing redox-active LBL films containing DNA.[6]

The present paper reports the preparation and the redox properties of LBL thin films composed of DNA and ferrocene-appended poly(allylamine) (Fc-PAH) or poly(ethyleneimine) (Fc-PEI). Ferrocene (Fc) has been widely used for developing redox-active organic materials because of its versatility in modification of the chemical structure.[7] In the present study, we report redox properties of the Fc-PAH/DNA and Fc-PEI/DNA LBL films deposited on the surface of a gold (Au) electrode, as a function of the number of layers in the LBL films. It has been found here that the redox behavior is dependent on the type of polycations used and the thickness of the film. The systematic study on the redox properties of redox-active DNA films may provide a basis for developing high-performance electrochemical sensors for detecting DNA intercalators and/or for screening DNA-binding drugs, because the redox properties of thin films are often quite sensitive to the changes in fine structures.

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Experimental Section

Materials. Calf Thymus DNA (double stranded) was purchased from Sigma Chemical Co. (St. Louis, USA). An aqueous solution (20%) of poly(allylamine hydrochloride) [PAH; average molecular weight (MW), ∼10 000] was purchased from Nittobo Co. (Tokyo, Japan). A 30% aqueous solution of poly(ethylenimine) (PEI; MW, 60 000−80 000) was obtained from Nakalai Tesque Co. (Kyoto, Japan). Ferrocenecarboxyaldhyde and sodium 3-mercapto-1-propanesulfonate (MPS) were obtained from Aldrich Chemical Co. (Milwaukee, WI) and Tokyo Kasei Co. (Tokyo, Japan), respectively. All of the materials were of the highest grade available and were used as received. The ferrocene-appended PAH and PEI (Fc-PAH and Fc-PEI) were synthesized using ferrocenecarboxyaldehyde and the poly(amine)s as reportedly.[8] The content of Fc moiety in Fc-PAH and Fc-PEI was determined to be 7.2 and 2.3 mol % (molar ratio of Fc to primary amine groups), respectively, by UV−visible absorption spectrometry. The chemical structures of Fc-PAH and Fc-PEI are shown in Figure 1.

Preparation of LBL DNA Films. A gold (Au) disk electrode (diameter, 3.0 mm) was used throughout. The surface of the electrode was first polished thoroughly with aqueous slurries of alumina paste and then sonicated in water. A clean surface of the electrode was obtained by a potential sweep from −0.2 to +1.7 V vs Ag/AgCl in a 0.5 M H_2SO_4 at the scan rate of 10 V s^{-1} for 15 min. The Au electrode was dipped in a freshly prepared 10 mM MPS aqueous solution overnight and then rinsed with water. The MPS-coated electrode was modified with LBL film by immersing it in a 0.1 mg mL^{-1} Fc-PAH or Fc-PEI solution (10 mM phosphate buffer containing 100 mM KCl, pH 7.0) for 15 min and, after being rinsed for 10 min in a distilled water, a 0.1 mg mL^{-1} DNA solution (10 mM phosphate buffer containing 100 mM KCl, pH 7.0) for 15 min, successively. The deposition of the Fc-bearing poly(amine)s and DNA was repeated for preparing Fc-PAH/DNA and Fc-PEI/DNA multilayer films.

For UV−visible and circular dichroism (CD) spectroscopies, the DNA film was prepared on the surface of a quartz slide (50 × 10 × 1 mm^3). The surface of the quartz slide was cleaned with a mixture of sulfuric acid and chromic acid, and then treated with a 10% dichlorodimethylsilane or γ-(aminopropyl)triethoxysilane solution in toluene overnight to make the surface hydrophobic or positively charged. The Fc-PAH/DNA and Fc-PEI/DNA films were deposited on the quartz slide in a similar procedure as in the deposition on the electrode. UV−visible absorption spectrometer (UV-3100PC, Shimazu, Japan) and CD spectrometer (J720, JASCO, Japan) were used for recording UV−visible and CD spectra.

For gravimetric measurements of the DNA films, the films were deposited on a 9 MHz At-cut quartz resonator coated with a thin Au layer, whose surface had been modified with a MPS monolayer before depositing DNA films. The deposited amounts of DNA and poly(amine)s were evaluated from changes in the resonance frequency of the quartz resonator using a quartz crystal microbalance (QCM) (QCA 917 system, Seiko EG & G, Tokyo, Japan). The adsorption of one ng of substance on the quartz resonator used induces a −0.91 Hz change in the resonance frequency.[9] For obtaining the gravimetric data, the probe was dried in air until the frequency showed a steady-state value. All data were obtained in air for dry films.

Electrochemical Measurements. Electrochemical measurements of the DNA film-coated electrode were carried out in a conventional three-

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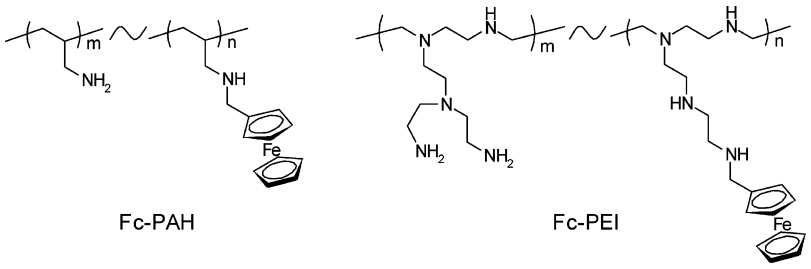

Figure 1. Chemical structures of Fc-PAH and Fc-PEI.

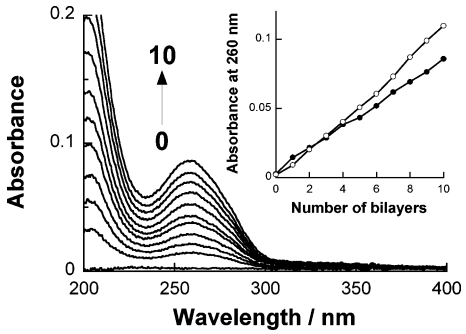

Figure 2. Typical UV−vis absorption spectra of (Fc-PAH/DNA)n LBL films. (Inset) Absorbance of the (Fc-PAH/DNA)$_n$ (○) and (Fc-PEI/DNA)$_n$ films (●) at 260 nm as a function of the number of bilayers.

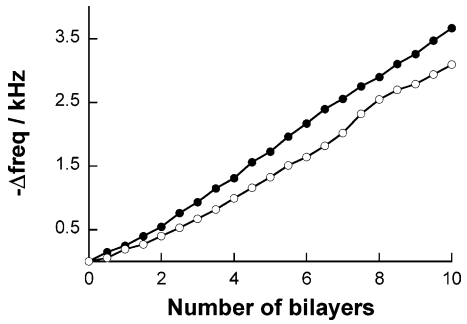

Figure 3. Typical frequency changes in QCM for the formation of (Fc-PAH/DNA)$_n$ (○) and (Fc-PEI/DNA)$_n$ films (●). The layer numbers between each integer show the deposition of Fc-PAH or Fc-PEI.

electrode system using a platinum wire as the counter electrode and a Ag/AgCl electrode as the reference electrode. All measurements were performed at room temperature using an electrochemical analyzer (660B, BAS).

Results and Discussion

Preparation of LBL Films Composed of DNA and Fc-PAH or Fc-PEI. Prior to electrochemical measurements of LBL films, the deposition behavior of the (Fc-PAH/DNA)$_n$ and (Fc-PEI/DNA)$_n$ films was evaluated using UV−visible absorption spectroscopy and QCM. Figure 2 shows UV−visible absorption spectra of the (Fc-PAH/DNA)$_n$ LBL films prepared on the surface of a quartz slide. The spectra exhibited an absorption maximum around 260 nm originating from DNA. The (Fc-PEI/DNA)$_n$ films showed almost the same absorption spectra. The inset plots the absorbance of the films at 260 nm as a function of the number of bilayers in the (Fc-PAH/DNA)$_n$ and (Fc-PEI/DNA)$_n$ films. The absorbance increased linearly with the increasing number of bilayers, suggesting that a constant amount of DNA is deposited in each deposition. It is reasonable to assume that the driving force of the film formation stems from an electrostatic force of attraction between phosphate residues in the DNA chain and the positively charged poly(amine)s.

QCM was also employed for evaluating the deposition behavior of the DNA films. Figure 3 plots the changes in resonance frequency ($-\Delta F$) upon deposition of DNA and Fc-poly(amine)s on the quartz resonator. The data shows that, for both (Fc-PAH/DNA)$_n$ and (Fc-PEI/DNA)$_n$ films, $-\Delta F$ increased linearly with the increasing number of layers, suggesting a linear growth of the LBL films. From the QCM data, the deposited amounts of DNA in the 10 bilayers of (Fc-PAH/DNA)$_{10}$ and (Fc-PEI/DNA)$_{10}$ films are calculated to be 4.4×10^{-6} and 4.2×10^{-6} g cm^{-2}, respectively. The QCM data also shows that 5.6×10^{-6} g cm^{-2} of Fc-PAH and 4.2×10^{-6} g cm^{-2} of Fc-PEI are contained in the (Fc-PAH/DNA)$_{10}$ and (Fc-PEI/DNA)$_{10}$ films, respectively. It is possible to roughly estimate the thickness of the films in dry state from the QCM data by assuming the density of the materials to be 1.2 g cm^{-3}.[10] According to this assumption, the thickness of the LBL films can be estimated to be 8.3 nm for the (Fc-PAH/DNA)$_{10}$ film and 7.0 nm for the (Fc-PEI/DNA)$_{10}$ film. Thus, both the spectroscopic and gravimetric data clearly show that the LBL deposition of the Fc-poly(amine)s and DNA successfully gives multilayer thin films.

CD spectra of the films were recorded to estimate the conformation of DNA in the LBL films (see the Supporting Information). The CD spectra of the film exhibit a negative peak at 210 nm. The negative peak at 210 nm is ascribable to DNA itself not originating from an induced CD of Fc moiety in the polymers, because almost the same CD spectrum was observed for the LBL film prepared using unmodified PAH in place of Fc-PAH under the same experimental conditions. It is known that DNA assumes the B form in an aqueous solution and exhibits a negative peak at 245 nm and a positive peak at 274 nm. Thus, the CD profile for the films is different from that in solution, suggesting a different conformation in the films. Lang and Liu also reported a CD spectrum of a PAH/DNA LBL film exhibiting a strong negative peak at 210 and a broad peak at 260 nm. They found that the DNA still binds ethidium bromide in the film and the original B form is restored, suggesting DNA assumes the conformation to which ethidium bromide can be intercalated in the film.[4a] Horinaka and co-workers have recently observed conformational changes of DNA upon complexation with PEI at the solid−liquid interface.[11] These observations suggest that the conformation of DNA in the Fc-PAH/DNA and Fc-PEI/DNA films is somewhat different from that in solution, but the helical conformation may still be preserved.

Redox Properties of Fc-PAH/DNA and Fc-PEI/DNA Films. It has been reported that LBL films containing Fc residues exhibit redox response when being deposited on the surface of an electrode.[12] In a previous study, we have prepared LBL films composed of poly(vinyl sulfate) (PVS) and Fc-PAH or Fc-PEI, in which the redox properties significantly depended on the type of the Fc-bearing poly(amine)s and the film thickness.[8] However, to the best of our knowledge, no report has appeared on the redox property of LBL film containing Fc and DNA. Therefore, it is interesting to study the redox property

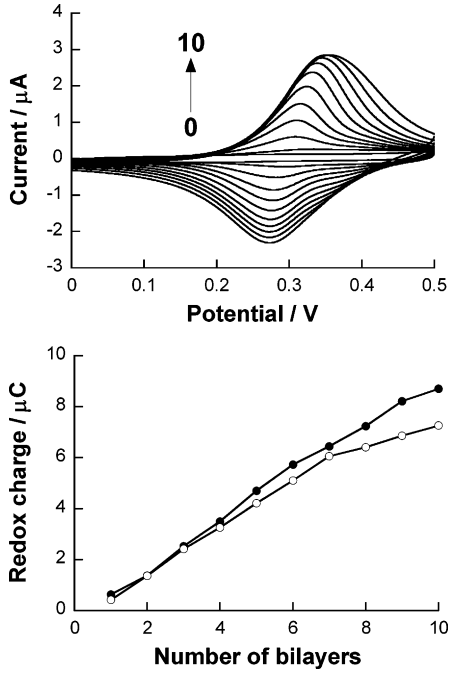

Figure 4. (Top panel) Cyclic voltammograms of (Fc-PAH/DNA)$_{n-1}$-Fc-PAH film-coated electrodes. (Bottom panel) Redoc charges of (Fc-PAH/DNA)$_{n-1}$Fc-PAH (○) and (Fc-PAH/DNA)$_n$ film-coated electrode (●). Scan rate; 50 mV s^{-1}.

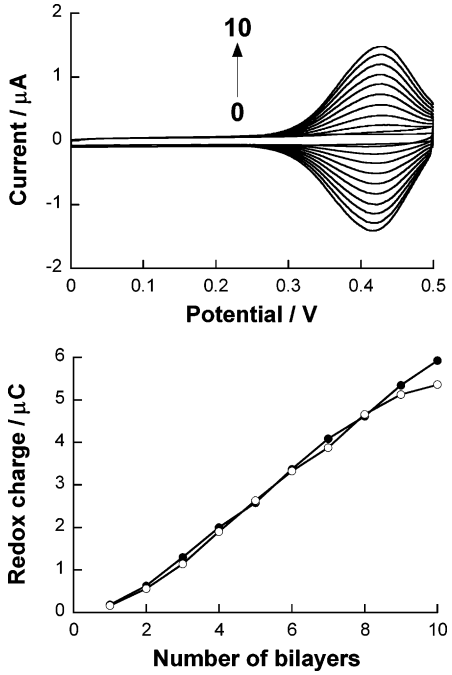

Figure 5. (Top panel) Cyclic voltammograms of (Fc-PEI/DNA)$_{n-1}$-Fc-PEI film-coated electrodes. (Bottom panel) Redoc charges of (Fc-PEI/DNA)$_{n-1}$Fc-PEI (○) and (Fc-PEI/DNA)$_n$ film-coated electrode (●). Scan rate; 50 mV s^{-1}.

of the Fc-PAH/DAN and Fc-PEI/DNA thin films prepared by the LBL technique.

Figure 4 depicts cyclic voltammograms (CV) of a (Fc-PAH/DNA)$_n$Fc-PAH film-coated electrode measured in 10 mM phosphate buffer containing 100 mM KCl (pH 7.0). The CV exhibited redox peaks originating from Fc residues in the LBL films, the peak current and redox potentials being dependent on the number of layers. The peak current increased with the increasing number of bilayers, implying that Fc residues in the outer layers are still electroactive as well as those located in the inner layers. The peak separation between the anodic and cathodic peaks (ΔE_p) also depends on the number of bilayers; the ΔE_p value for the (Fc-PAH/DNA)$_3$Fc-PAH film-coated electrode is ca. 55 mV, whereas the value is ca. 85 mV for the (Fc-PAH/DNA)$_8$Fc-PAH film. It is known that the CVs for the surface-confined redox species exhibit a symmetric shape (or the anodic and cathodic peak potentials are identical with each other, $\Delta E_p = 0$ V), whereas the diffusion controlled redox systems afford $\Delta E_p = 60$ mV in the reversible case. For the thicker (Fc-PAH/DNA)$_n$Fc-PAH film-coated electrodes, the redox reaction is apparently diffusion controlled. Another feature of the CV is that the redox properties depend on the type of the outermost layer. The bottom panel in Figure 4 plots the electric charge (Q) passed upon redox reaction of the Fc residues in the films. The Q values for the (Fc-PAH/DNA)$_n$Fc-PAH film-coated electrodes are slightly higher than those of the electrodes whose outermost surface is covered with DNA, even if both films contain the same number of Fc-PAH layers. In other words, the redox activity of the films depends on the polarity of the electric charges on the outermost surface of the film, which is often the case for redox-active LBL films.[12a,13] In fact, we have found a similar trend for (Fc-PAH/PVS)$_5$(PAH/PVS)$_n$ multilayer films, in which the Q values of the PAH-terminated films were always higher than those of the PVS-terminated films.[14] The higher response of the cationic PAH-terminated film may be ascribed in part to the facilitated anion transport through the film to compensate the positive charges of ferricinium cations. In other words, the diffusion of ions in the film may participate in the rate determining step of the redox reaction of the films. This view is consistent with the fact that the shape of CVs for the (Fc-PAH/DNA)$_n$Fc-PAH film-coated electrodes is similar to that for diffusion-controlled systems.

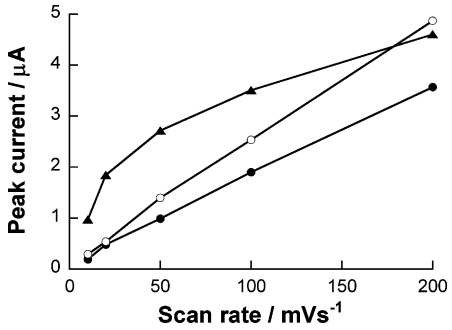

Figure 6. Peak current of CVs for (Fc-PEI/DNA)$_9$Fc-PEI (●), (Fc-PAH/DNA)$_2$Fc-PAH (○), and (Fc-PAH/DNA)$_9$Fc-PAH film-coated electrodes (▲) as a function of scan rate.

The redox properties of (Fc-PEI/DNA)$_n$Fc-PEI film-coated electrodes are quite different from those of the electrodes coated with the Fc-PAH-based films. Figure 6 illustrates CVs for the (Fc-PEI/DNA)$_n$Fc-PEI film-coated electrodes. For all films, the peak-to-peak separation is 10 mV or less, confirming that the redox waves mainly originate from surface-confined redox species. The bottom panel shows the Q values for the (Fc-PEI/DNA)$_n$Fc-PEI and (Fc-PEI/DNA)$_n$ film-coated electrodes as a function of the number of Fc-PEI layers, where the Q value was proportional to the number of Fc-PEI layers. The Q value for the LBL films containing the same number of Fc-PEI layers is almost the same with each other regardless of the sign of the electric charge in the outermost layer, in contrast to the significant effects of the outermost layer observed for the Fc-PAH-based LBL films (Figure 4). These observations suggest that no diffusion process participates in the rate determining step of the redox reactions in the (Fc-PEI/DNA)$_n$Fc-PEI and (Fc-PEI/DNA)$_n$ film-coated electrodes. It is likely that the redox

response of the films stems from an electron hopping between an oxidized and an adjacent reduced form of Fc residues in the film in view of the fact that all ferrocene residues are attached to the polymer chains and, consequently, the translocational diffusion is not plausible.[15]

We have separately measured CVs for the electrodes coated with Fc-free $(PEI/DNA)_n$ and $(PAH/DNA)_n$ films and found no redox peak in the potential range of 0−0.5 V. These observations clearly show that the redox peaks for the films containing Fc-PEI and Fc-PAH originate not from DNA bases such as guanine and adenine but from the Fc moieties in the films.

It is known that the peak current of CV increases linearly with the scan rate or with the square root of the scan rate, depending on the electron transport mechanism on the electrode. Figure 6 plots the anodic peak current of the $(Fc\text{-}PAH/DNA)_2$Fc-PAH, $(Fc\text{-}PAH/DNA)_9$Fc-PAH, and $(Fc\text{-}PEI/DNA)_9$Fc-PEI film-coated electrodes as a function of the scan rate of CV. The peak current of the $(Fc\text{-}PEI/DNA)_9$Fc-PEI film-coated electrode exhibited a linear dependence on the scan rate in the range of 10−200 mV s^{-1}, confirming the surface-confined species as an origin of the redox reaction. For the Fc-PAH-based films, a linear relationship was observed for the thinner $(Fc\text{-}PAH/DNA)_2$Fc-PAH film while not for the thicker film. Thus, the mechanism of electron transport in the LBL film depends on the type of poly(amine)s used and on the film thickness; a diffusion-controlled process is involved in part in the Fc-PAH-based thicker film while not in the Fc-PEI-based film. We have previously observed a similar behavior for the Fc-PAH/PVS and Fc-PEI/PVS LBL films.[8] Dominguez and co-workers also observed a transition of the electron transport mechanism depending on the thickness of the LBL films containing osmium bipyridyl complex.[16] The different behavior between the Fc-PAH- and Fc-PEI-based LBL films may be ascribed to the different ion-permeability of the films, which in turn originates from the different molecular geometry of the polymer chains of Fc-PAH and Fc-PEI. As illustrated in Figure 1, PAH used is a linear polymer without any branching, whereas PEI is a highly branched polymer containing primary, secondary, and tertiary amino groups in the ratio of ca. 1:2:1. We have previously found that the permeability of the LBL films containing PAH is different from that of the PEI-based LBL films. In general, the permeability of PAH-based LBL films is significantly lower compared to the highly permeable nature of PEI-based films, probably due to the different packing of the polymer chains in the LBL films.[17] The polymer chains may be densely packed in PAH-based LBL films, whereas the packing is rather loose in PEI-based LBL films. Amino groups in Fc-PAH may form ion pairs complementarily with phosphate anions on the DNA, resulting in a compact film in which Fc-PAH and DNA chains are densely packed. In this situation, in the thicker $(Fc\text{-}PAH/DNA)_9$Fc-PAH film, an ion transport may be slow and thus involved in the rate determining step in the redox reaction in the film. The cationic charges of the ferricinium ion should be compensated by ion transport in and/or out of the film to hold an electric neutrality. In contrast, Fc-PEI and DNA may form a loosely packed film due to a geometrical mismatching of the highly branched Fc-PEI and double-stranded chains of DNA.

It was found that a significant portion of the Fc residues in the film is not involved in the redox reaction under the present experimental conditions. Figure 7 plots the ratio of redox active Fc residues in the films as a function of the number of bilayers, which was estimated on the basis of the *Q* values compared with the Fc loading in the films estimated by QCM. The redox-active Fc residues in the $(Fc\text{-}PAH/DNA)_n$ and $(Fc\text{-}PAH/DNA)_n$Fc-PAH films are 20−25%, whereas for the $(Fc\text{-}PEI/DNA)_{10}$ film, a similar calculation shows that 40−60% of Fc residues are redox active. We have previously found that, in the case of the $(Fc\text{-}PAH/PVS)_{10}$ film, 56% of the Fc residues are redox active.[8] The electron hopping in the DNA films may be significantly disturbed by the DNA chains which assume a bulky conformation in the film, as compared to a smooth electron transport in the $(Fc\text{-}PAH/PVS)_{10}$ LBL film due to a flexible polymer chain of PVS. This effect is more significant in the Fc-PAH/DNA films than in the Fc-PEI/DNA films probably due to a tight packing of the polymer chains in the Fc-PAH/DNA films.

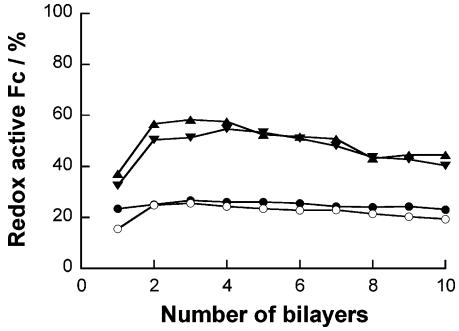

Figure 7. Ratios of redox active Fc residues in $(Fc\text{-}PAH/DNA)_{n-1}$-Fc-PAH (○), $(Fc\text{-}PAH/DNA)_n$ (●), $(Fc\text{-}PEI/DNA)_{n-1}$Fc-PEI (▲), and $(Fc\text{-}PEI/DNA)_n$ films (▼).

Conclusion

The redox-active thin films containing DNA can be successfully prepared by an alternate deposition of Fc-PAH or Fc-PEI and DNA on the surface of a quartz slide and on a Au electrode. The LBL film-modified electrodes exhibited a redox response although a significant portion of the Fc moieties in the film was electrochemically inactive probably due to hindered electron hopping by the bulky DNA layer. The redox properties of the LBL films depended on the type of the polymer chains and on the film thickness. The Fc-PEI/DNA film-coated electrode showed CVs ascribable to the surface-confined redox species, whereas the mechanism of electron transport in the Fc-PAH/DNA films was dependent on the thickness of the film. The DNA-containing LBL films may be useful for constructing electrochemical sensors for detecting toxic compounds or drugs that can bind to DNA. Such compounds may induce changes in the electrochemical response of the LBL film-modified electrodes.

Supporting Information Available. CD spectra of $(DNA/Fc\text{-}PAH)_9$DNA (A), $(DNA/Fc\text{-}PEI)_9$DNA (B), and $(DNA/PAH)_9$DNA (C) films on the surface of the quartz slide. This material is available free of charge via the Internet at http://pubs.acs.org.

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