Electrochemically Induced Disintegration of Layer-by-Layer-Assembled Thin Films Composed of 2-Iminobiotin-Labeled Poly(ethyleneimine) and Avidin

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A layer-by-layer assembly composed of avidin and 2-iminobiotin-labeled poly(ethyleneimine) (ib-PEI) was prepared on the surface of a platinum (Pt) film-coated quartz resonator, and an electrochemically induced disintegration of the avidin−ib-PEI assembly was studied using a quartz crystal microbalance. The resonance frequency of a five-bilayer (avidin−ib-PEI)$_5$ film-coated quartz resonator was increased upon application of an electric potential to the Pt layer of the quartz resonator, suggesting that the mass on the quartz resonator was decreased as a result of disintegration of the (avidin−ib-PEI)$_5$ film, due to a pH change in the vicinity of the surface of the Pt-coated quartz resonator. It may be that the (avidin−ib-PEI)$_5$ film assembly was decomposed by acidification of the local pH on the surface of the Pt layer, which in turn was induced through electrolysis of water on Pt, because ib-PEI forms complexes with avidin only in basic media. In pH 9 solution, the (avidin−ib-PEI)$_5$ film was decomposed under the influence of an applied potential of 0.6−1.0 V versus Ag/AgCl. The (avidin−ib-PEI)$_5$ film was decomposed almost completely within a minute in a low concentration buffer (1 mM, pH 9), while the decomposition was slower in 10 and 100 mM buffer solutions at the same pH. The decomposition of the assembly was rapid when the electrode potential was applied in pH 9 solutions, while the response was relatively slow in pH 10 and 11 solutions. All the results are rationalized on the basis of an electrochemically induced acidification of the local environment around the (avidin−ib-PEI)$_5$ film on the Pt layer.

Introduction

A layer-by-layer (LbL) deposition technique has been widely used for preparing layered thin films by taking advantage of the electrostatic force of attraction,[1] hydrogen bonding,[2] and biological affinity[3] between synthetic polymers,[4] proteins,[5] DNA,[6] polypeptides,[7] and so forth. Binding proteins such as antibody,[8] lectin,[9] and avidin[10] have also been employed for constructing layered nanoassemblies through a biological affinity. The layered thin films have found applications in optical and electrochemical devices,[11] biosensors,[12] encapsulation, and controlled release.[13]

Heretofore, much attention has been devoted to the development of durable films that are stable in the media where the layered films are used. In contrast to this concept, Sukhishvili and Granick recently showed that layered thin films composed of poly(methacrylic acid) and poly(vinylpyrrolidone) can be disintegrated by a change in the environmental pH due to suppressed hydrogen bonding.[14] The NaCl-induced degradation of LbL thin films and hydrolytically degradable polymer films was also reported for controlled release.[15,16] In this context, we recently reported that LbL thin films composed of concanavalin A (Con A) and glycogen are sensitive to sugars and are degradable in response to D-glucose and other sugars.[17,18] A Con A/dextran assembly has been used for the fluorometric determination of D-glucose.[19] We have also reported that LbL thin films composed of avidin can be disintegrated by changing the environmental pH or by being exposed to biotin and derivatives.[20,21] The avidin-containing films were prepared using 2-iminobiotin-labeled poly(ethyleneimine) (ib-PEI) and avidin by taking advantage of the biological affinity between 2-iminobiotin and avidin.

Avidin is a glycoprotein (molecular weight, 68 000) found in egg white and is known to contain four binding sites identical to those of biotin (binding constant, $\sim 10^{15}$ M^{-1}).[22] It is also known that avidin binds 2-iminobiotin less strongly than biotin, and the affinity is pH dependent (the binding constant of 2-iminobiotin to avidin is 2.9×10^{10} M^{-1} in a basic solution, while the value of the protonated form in acidic media is $\sim 10^3$ M^{-1}).[23] It is thus reasonable to assume that the LbL films prepared using avidin and ib-PEI are sensitive to the environmental pH. In fact, we have found that the avidin−ib-PEI multilayer films can be completely decomposed by changing the environmental pH from basic to weakly acidic.[20,21] These findings have prompted us to study the effects of electrode potential on the stability of the avidin−ib-PEI films because the local pH in the vicinity of the electrode surface may be altered upon changing the potential of the electrode where avidin−ib-PEI films are coated. The present communication reports that avidin−ib-PEI LbL films prepared on the surface of a platinum (Pt)-coated quartz resonator can be disintegrated upon applying an electric potential to the Pt layer probably because of an acidification of the local pH around the Pt surface (Figure 1). Recently, Boulmedais and co-workers have reported on the electrochemically induced disintegration of LbL films for controlled drug delivery.[24]

Experimental Section

Materials. Avidin was purchased from Calzyme Lab., Inc. ib-PEI was prepared by the reaction of PEI (from Nakalai Tesque Co., Japan;

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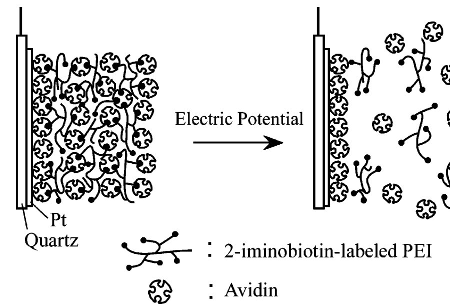

Figure 1. Disintegration of an (avidin−ib-PEI) LbL film induced by the application of electric potential.

molecular weight, 60 000−80 000) and 2-iminobiotin *N*-hydroxysuccinimide ester hydrobromide (Sigma Co., St. Louis, MO) according to the reported procedure (the content of 2-iminobiotin residues in the ib-PEI is ~2 mol % based on the total amino groups in PEI).[20,21] Other reagents used were of the highest grade available and used without further purification.

Apparatus. A quartz crystal microbalance (QCM) (QCA 917 system, Seiko EG & G, Tokyo, Japan) was employed for the gravimetric analysis of the LbL thin films. A 9-MHz AT-cut quartz resonator coated with a thin Pt layer (surface area, 0.2 cm^2) was used as a probe, in which the adsorption of 1 ng of substance induces a −0.91 Hz change in the resonance frequency.

Preparation of Avidin−ib-PEI LbL Films. The avidin−ib-PEI LbL films were deposited on a Pt film-coated quartz resonator to monitor the resonance frequency using a QCM. The quartz resonator was mounted into a measuring cell and immersed in an avidin solution (0.1 mg mL^{-1}) for 15 min at room temperature to deposit the first layer of avidin. After being rinsed in the working buffer for 5 min, the quartz resonator was immersed in an ib-PEI solution (0.1 mg mL^{-1}) for 15 min to deposit the ib-PEI. The deposition was repeated in order to build up the multilayer assemblies. A 10 mM borate buffer containing 100 mM KCl (pH 11) was used to prepare the avidin and ib-PEI solutions.

Disintegration of Avidin−ib-PEI LbL Films. The disintegration of the avidin−ib-PEI LbL films was studied by monitoring the resonance frequency of the five-bilayer $(avidin-ib-PEI)_5$ film-coated quartz resonator with QCM in borate buffer solutions containing 100 mM KCl.

Before applying the electrode potential, a steady-state value of the resonance frequency of the $(avidin-ib-PEI)_5$ film-coated quartz resonator was recorded in a buffer solution. Then, the changes in the resonance frequency were monitored upon applying the electric potential to the Pt layer on the resonator, using a potentiostat (NPGF-2500, Nikkou Keisoku, Atsugi, Japan). A Ag/AgCl electrode (3 M KCl) was used as a reference electrode. All measurements were carried out at room temperature (~20 °C).

Results and Discussion

Figure 2 shows changes in the resonance frequency (ΔF) of QCM observed for the deposition of five-bilayer $(avidin-ib-PEI)_5$ film on the quartz resonator and an electrochemically induced disintegration of the film. The surface of a Pt-coated quartz resonator was first exposed to avidin and ib-PEI solutions (pH 11) alternately to prepare an $(avidin-ib-PEI)_5$ LbL film on the quartz resonator. The resonance frequency was decreased upon deposition of both avidin and ib-PEI, suggesting that the $(avidin-ib-PEI)_5$ LbL film was successfully deposited on the quartz resonator. The driving force of the formation of the LbL film is a strong affinity between the avidin and 2-iminobiotin residues in ib-PEI in the basic media (binding constant, 2.9×10^{10} M^{-1}).[20,21] From the QCM results, the loading of the $(avidin-ib-PEI)_5$ LbL film was calculated to be ~1.0×10^{-5} g cm^{-2}. Consequently, the thickness of the five-bilayer $(avidin-ib-PEI)_5$ film is estimated to be 80 ± 3 nm in the dry state, assuming that the density of the film is ~1.2−1.3 g cm^{-3}.[25]

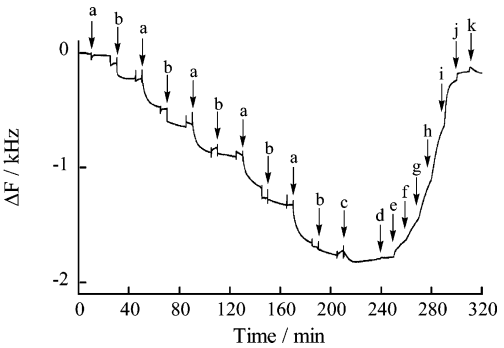

Figure 2. The deposition and disintegration of an $(avidin-ib-PEI)_5$ film monitored with QCM. The Pt-coated quartz resonator was immersed in 0.1 mg mL^{-1} avidin (a) and ib-PEI (b) in 10 mM borate buffer solutions (pH 11) alternately to deposit the $(avidin-ib-PEI)_5$ film. During the preparation of the LbL film, the film was rinsed in the working buffer between the depositions of avidin and ib-PEI. For the disintegration of the film, the $(avidin-ib-PEI)_5$ film-modified quartz resonator was immersed in 1 mM borate buffer (pH 9.0) at c, and the electric potential was applied to the Pt layer. Potential: 0 (d), 0.6 (e), 0.7 (f), 0.8 (g), 0.9 (h), 1.0 (i), 1.1 (j), and 1.2 V vs Ag/AgCl (k).

The $(avidin-ib-PEI)_5$ film-coated quartz resonator was then immersed in 1 mM borate buffer (pH 9) to evaluate the effects of electrode potential on the stability of the film. It is known that the $(avidin-ib-PEI)_5$ film is stable in pH 9 or more basic media.[20,21] An electrode potential was applied to the Pt layer of the quartz resonator, and the resonance frequency of the $(avidin-ib-PEI)_5$ film-coated quartz resonator was monitored. Virtually no effect was observed upon applying 0 V, while the resonance frequency increased when 0.6 V or more positive potential was applied. Upon applying 1.1 V to the quartz resonator, the ΔF value reached back to ~0, suggesting that the mass on the quartz resonator was removed almost completely as a result of the disintegration of the $(avidin-ib-PEI)_5$ film. In other words, the $(avidin-ib-PEI)_5$ film may be fully fragmented into the components avidin and ib-PEI and dissolved in the buffer solution. This is probably due to the local pH change induced by the electrolysis of water in the vicinity of the surface of the Pt layer. It is reasonable to assume that the pH around the Pt surface shifts in the acidic direction because a positive potential was applied to the Pt layer, which should function as an anode to oxidize water. In fact, it has been reported that the electrolysis of water results in an acidification of the local pH at the anode.[26−30] Thus, it is likely that the binding ability of the 2-iminobiotin residues in ib-PEI to avidin was significantly lowered in the acidic environment, and the $(avidin-ib-PEI)_5$ film was decomposed into avidin and ib-PEI, as in the case of the decomposition of the film induced by pH changes in the media.[20,21]

The effects of the applied potential on the disintegration of the $(avidin-ib-PEI)_5$ film were evaluated by applying the potential from 0.6 to 1.0 V versus Ag/AgCl separately. The resonance frequency of the $(avidin-ib-PEI)_5$ film-coated quartz resonator was monitored in 1 mM borate buffer (pH 9) under the influence of the electrode potential from 0.6 to 1.0 V (Figure 3). The decomposition behavior clearly depended on the magnitude of the electrode potential; the decomposition was more rapid under the higher potential, supporting the idea that the decomposition is induced by the electrode potential applied to the Pt layer. In a separate measurement, it was found that the effects of the lower potential (0−0.5 V) are very small.

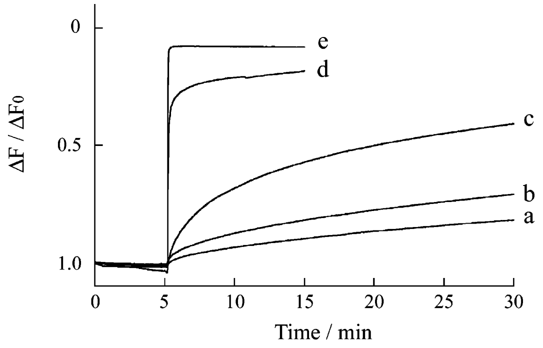

Figure 3. QCM frequency changes of the (avidin−ib-PEI)$_5$ film-coated quartz resonator induced by the application of electric potential from 0.6 to 1.0 V in 1 mM borate buffer solution (pH 9.0). ΔF_0 denotes the frequency for the (avidin−ib-PEI)$_5$ film before disintegration, while ΔF shows the values at each time. The electrode potential: 0.6 (a), 0.7 (b), 0.8 (c), 0.9 (d), and 1.0 V (e).

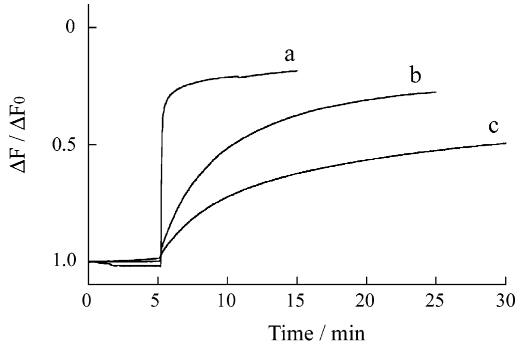

Figure 4. QCM frequency changes of the (avidin−ib-PEI)$_5$ film-coated quartz resonator induced by the application of electric potential (0.9 V) in 1 (a), 10 (b), and 100 mM (c) borate buffer solutions at pH 9.0.

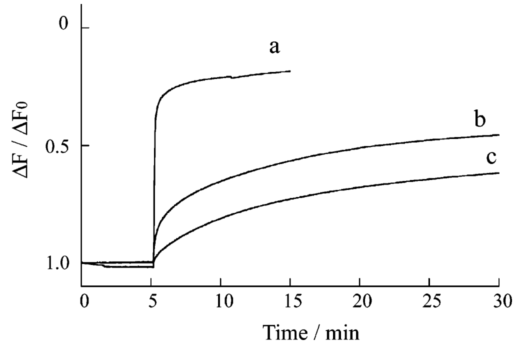

Figure 5. QCM frequency changes in the (avidin−ib-PEI)$_5$ film-coated quartz resonator induced by the application of electric potential (0.9 V) in 1 mM borate buffer solutions at pH 9.0 (a), 10 (b), and 11 (c).

Figure 4 shows the effects of the concentration of buffer on the decomposition of the (avidin−ib-PEI)$_5$ film. A 0.9 V potential was applied to the Pt layer in 1, 10, and 100 mM borate buffers at pH 9. The film was decomposed rapidly when the electrode potential was applied in 1 mM buffer solution, while the decomposition rates were slower in 10 and 100 mM buffer solutions. The slower response in the 10 and 100 mM buffer solutions implies that the electrochemically induced pH change was effectively suppressed in the 10 and 100 mM buffer solutions but not in the 1 mM buffer due to the lower buffer capacity. These observations further support the view that the decomposition of the film was triggered by a pH change around the Pt surface.

Figure 5 illustrates the effects of pH of the solutions wherein the film was immersed. The QCM measurement was carried out in 1 mM borate buffer solutions at pH 9, 10, and 11. The (avidin−ib-PEI)$_5$ film was decomposed rapidly upon application of the electrode potential in the pH 9 solution, whereas, in pH 10 and 11 solutions, the film was decomposed rather slowly. In the pH 9 solution, the true pH around the film may rapidly shift to pH 8 or a more acidic value under the influence of the applied potential. It has been shown that a threshold pH for the decomposition of the (avidin−ib-PEI)$_5$ film is ~8.0.[20,21]

Conclusions

All data reported herein strongly suggest that the (avidin−ib-PEI)$_5$ film deposited on the surface of a Pt-coated quartz resonator can be decomposed in response to the electrode potential applied to the Pt layer. It is probable that the decomposition of the film is attributable to the acidification of the local pH in the vicinity of the Pt layer, which originates from the electrolysis of water at the surface of the Pt layer poised at positive potentials. It should be noted here that an application of positive potential suffices to decompose the film, and no reagent is required. This is a clear contrast to other degradable systems that need chemical reagents such as small ions and molecules, acids, and bases to induce degradation.[14,31−34] The present reagentless system would be useful in view of the practical applications of the film in separation and purification, controlled release, and other purposes.

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