Controlled Desorption of Films

Time-Controlled Desorption of Ultrathin Polymer Films Triggered by Enzymatic Degradation**

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The fabrication of ultrathin polymer films on materials modifies the intact surface characteristics without changing the bulk properties. Time-controlled desorption of polymers from ultrathin films deposited on materials is an essential requirement for the time control of the surface characteristics and for the controlled release of substances from materials.

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These properties are especially important for applications in biomedical devices. The regulation of assembled polymer structures at the molecular level, as well as the suitable selection of polymer constituents, enables the systematic design and construction of tailored polymer architecture. It is, however, difficult to achieve these requirements by conventional methodologies.

The most attractive method for the fabrication of multilayered ultrathin polymer films is layer-by-layer (LbL) assembly, which is simply performed by repetitive alternate immersion of materials into interactive polymer solutions.[1] This method does not restrict the material shapes in the LbL assembly. The number of steps in the LbL assembly permits easy control of the thickness of the polymer to a molecular level. LbL assembly is applicable to all interactive polymer combinations, including synthetic and biopolymers. Not only electrostatic interactions but also other interactions between polymers have been utilized for the LbL assembly of functional polymers.[2] The LbL assembly method potentially could be used in applications involving time-controlled desorption of polymer constituents. Several reports have already demonstrated deconstruction (or simple desorption) of LbL assemblies that depend on the preparation conditions and/or the immersion media.[3]

Suitable combinations of biopolymers and enzymes, which hydrolyze the corresponding biopolymers in the LbL assembly, permit development of a model system that demonstrates controlled desorption of ultrathin polymer films. We previously reported desorption of the LbL assembly between cationic chitosan and anionic dextran sulfate through the hydrolysis of chitosan by chitosanase.[4] When the solution temperature was raised to a temperature at which the enzyme was active (40 °C), after electrostatic condensation of the cationic enzyme on the outermost surface of dextran sulfate at 4 °C, the multilayered film was immediately hydrolyzed. Conversely, the film with an outermost surface composed of chitosan was not hydrolyzed. While the bioactivity of each alternating layer of the LbL assembly has been demonstrated, time-controlled desorption has not. The results from using the system also suggested that it is not necessary for all the polymers to be hydrolyzed by enzymes for desorption of the LbL assembly to occur.

To generate the present model system, we have carried out the LbL assembly by using a combination of an anionic biopolymer DNA, a cationic synthetic polymer poly(diallyldimethylammonium chloride) (PDDA), and the endonuclease deoxyribonuclease I (DNase I) as a single-component-specific enzyme, which can be electrostatically adsorbed onto the PDDA surface. Not only differences in the activity of the alternating layers of the enzymatic hydrolysis of the LbL assembly but also time-controlled desorption on the basis of enzymatic hydrolysis are expected, since the activity of DNase I is strongly dependent on the concentration of essential metal ions, Mg^{2+} and Ca^{2+}.[5] Both cations activate the phosphodiester of DNA, and Ca^{2+} ions coordinate to DNase I to stabilize the structure. Accordingly, it is worthwhile to analyze whether enzymes already adsorbed onto the surface of the LbL assembly that are not denatured can hydrolyze DNA in the LbL assembly in response to changes

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in the ionic environment. The necessity for another polymer component corresponding to PDDA, which is not hydrolyzed by the enzyme, is also clearly demonstrated by the present system. Herein, we report the time-controlled desorption of an LbL assembly of DNA and PDDA on the basis of the enzymatic hydrolysis by DNase I (Figure 1), which is condensed on the PDDA surface and activated by metal ions in the media.

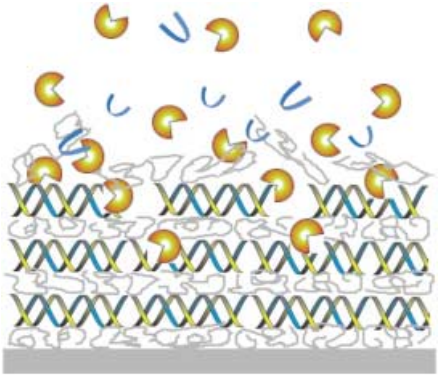

Figure 1. Schematic representation of the enzymatic hydrolysis of a DNA–PDDA assembly.

Researchers have already demonstrated that enzymes incorporated into the LbL assemblies on a porous filter membrane are active against small-molecule substrates that were applied across the membrane.[6] In the present case, the anionic charges of the DNA phosphodiester, which bind two metal ions, were used to provide electrostatic interactions with the quaternary amines of PDDA. Thus, for the coordinated interaction of PDDA with the phosphodiester, the metal ions would have to dissociate these ionic pairs. In other words, the dissociation could potentially be controlled by means of ion concentration. Furthermore, it is interesting to consider whether DNase I adsorbed onto the outermost surface can hydrolyze the DNA assembled inside the LbL structure. To achieve this, DNase I would have to progress to the inner layer by performing repetitive hydrolysis.

Before the demonstration of time-controlled desorption of the LbL assembly, differences in the activity of DNase I condensed onto the assembly were analyzed by using the 16- and 17-step assemblies with the respective surfaces of DNA and PDDA. Quantitative analysis by using a quartz crystal microbalance (QCM) as the assembly substrate revealed a clear difference in activity between the two assemblies (Figure 2). DNase I did not adsorb onto the DNA surface by substrate affinity, and desorption was not observed even after immersion into a buffer solution containing the essential ions. On the other hand, DNase I was electrostatically adsorbed onto the PDDA surface, since the enzyme was anionically charged in Tris buffer at pH 7.8 (the isoelectric point of the enzyme was 5.0). Subsequently, the assembly was clearly desorbed in the presence of Mg^{2+} and Ca^{2+} ions at concentrations of 50 mM for each ion. These observations suggest that DNase I electrostatically condensed on the PDDA surface became active through the binding of the

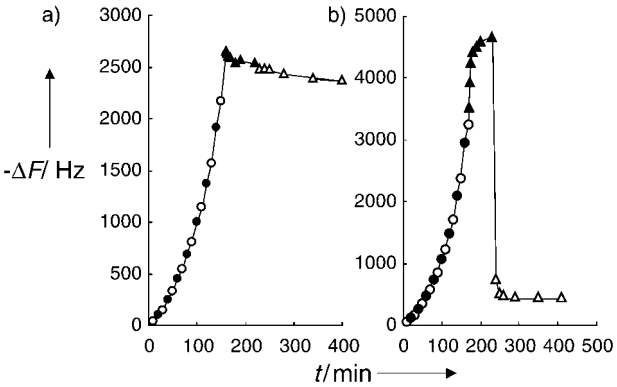

Figure 2. LbL assemblies of DNA and PDDA with outermost surfaces of a) DNA (16 steps) and b) PDDA (17 steps) and their hydrolyses by DNase I. Open and closed circles show the assembly steps of PDDA and DNA, respectively. The closed triangle shows the adsorption of DNase I at 4 °C. The subsequent open triangle shows desorption triggered by enzymatic degradation of the DNA in the assembly at 25 °C. $-\Delta F$ = Frequency shift.

ions to suitable sites. After fragmentation of the DNA, the assembly was desorbed by the formation of soluble polyion complexes with PDDA[7] or by dissociation of polyion complexes, which indicates that more than one component is required in the assembly to form a degradable polymer that desorbs by enzymatic hydrolysis. Note that the temperature required for condensation of the enzyme did not affect subsequent enzymatic activity.

The fragmentation of DNA solubilized in the reaction media was confirmed by the addition of the stain ethidium bromide, which inserts itself between double-stranded DNA base pairs at 590 nm and at ambient temperature, and is revealed by excitation at 260 nm. At 25 °C fluorescence was observed from the native DNA solution, and from the mixed solution containing DNA and PDDA, the concentrations of which were set at the same as those ideally desorbed based on the results of quantitative QCM analysis. However, at this temperature fluorescence was barely visible from the reaction media. This observation strongly suggests that DNase I fragmented the DNA in the assembly, which was followed by a decrease in the melting temperature (T_m) of the DNA. The T_m of the DNA desorbed was estimated to be between 4 °C and ambient temperature, since the fluorescence was observed from the reaction media at 4 °C (see Supporting Information). This observation indicates that the single-stranded DNA was desorbed from the assembly at a reaction temperature of 25 °C.

It is interesting to discuss whether DNase I was adsorbed on the PDDA surface of the 17-step assembly, or incorporated into the assembly. Atomic force microscopic (AFM) observation revealed that the mean roughness (Ra) of the assembly deposited on a mica substrate, the protein-adsorbed assembly, and the desorbed assembly were 3.0, 3.0, and 0.33 nm, respectively. The surface topology of the first two surfaces was clearly different, indicating the adsorption of enzymes (see Supporting Information). As suggested in previous reports, the charges that exist inside the LbL assemblies are compensated by electrostatic interactions.[8] Accordingly, the

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enzyme adsorbed on the surface seemed to hydrolyze the DNA in a stepwise fashion, thus progressing to the inner layer. Small amounts of the assembly as well as the enzyme remained even after sufficient reaction time, possibly because of difficulties associated with desorption (Figure 2b).

To demonstrate the time-controlled desorption, the 17-step assembly with PDDA on the outermost surface was hydrolyzed by the condensed DNase I under different concentrations of essential metal ions, based on the results of quantitative QCM analysis (Figure 3). While only the enzyme

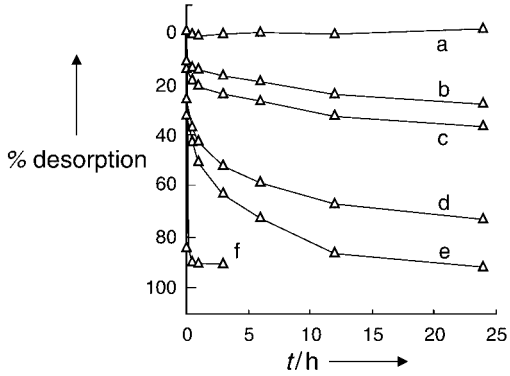

Figure 3. Desorption (%) of the 17-step LbL assembly with an outer surface composed of PDDA triggered by the enzymatic degradation of DNA in the assembly at 25 °C at concentrations of both Mg^{2+} and Ca^{2+} of a) 0, b) 0.5, c) 1, d) 3, e) 5, f) 50 mM after electrostatic condensation of the enzyme at 4 °C.

that was condensed on the polymer surface was desorbed in buffer solution, the degree of desorption increased with increasing ion concentrations. Mg^{2+} and Ca^{2+} ions clearly accelerated the hydrolysis of DNA, and the desorption could be controlled by altering the ion concentration. The presence of both types of ions was essential for desorption of the assembly; the assembly was not desorbed in the presence of only a single type of ion. Even in the presence of 100 mM NaCl there was no desorption of the LbL assembly, thus the specificity of the metal ions is demonstrated. Desorption was not complete even after sufficient reaction time had passed, and the amount that remained increased with decreased ion concentration. Since the charges of the DNA and the enzyme were already used for the LbL assembly process and electrostatic condensation onto the PDDA surface, respectively, replacement by ions, which is directly related to enzymatic activity, seemed to be dependent on ion concentration. The degree of desorption was also controllable by the adsorption time of the enzyme as well as enzyme concentration. In fact, an immersion of the assembly for 5 s in an enzyme solution of 0.05 mg mL^{-1}, a concentration which was a tenth of that used in the above measurements, resulted in approximately half the desorption at concentrations of both cations of 50 mM, and a netlike surface topology was observed by using AFM analysis (see Supporting Information). This result suggests that the present methodology enables three-dimensional regulation of the surfaces of the nanostructure of ultrathin polymer films. Notably, the enzyme incorporated inside the film composed of $(PDDA\text{-}DNA)_8$-PDDA-DNase I-PDDA-$(PDDA\text{-}DNA)_8$ and prepared by stepwise assembly did not degrade the DNA at concentrations of both cations of 5 mM, indicating that the ions did not diffuse into the film to allow enzymatic activity.

Time-controlled desorption of the LbL assembly was demonstrated by the electrostatic condensation of the enzyme that is specific for a single component of the assembly, and subsequent hydrolysis under suitable concentrations of ions essential for the enzyme. The enzyme was preserved on the surface of the LbL assemblies without being denatured, and was activated by external ionic stimuli. It would be interesting to imbed the enzymes into the LbL assemblies for further controlled desorption of the assembly. We are also currently investigating the application of the present desorption feature for the controlled release of substances from bulk materials.

Experimental Section

Materials: Salmon testis DNA (Wako, $\bar{M}_w$ 2–3 × 10^4 Da) and PDDA (Aldrich, $\bar{M}_w$ of 1–2 × 10^5) were used without further purification. DNase I (Nacalai, $\bar{M}_w$ 31 000) was dissolved in 10 mM Tris buffer at pH 7.8 before use.

LbL assembly: A 9 MHz QCM plate (USI System, Japan) with polished gold electrodes, and a diameter of 4.5 mm was used as the assembly substrate. The frequency shift (ΔF) was related to the amount of assembly (Δm) in accordance with the Sauerbrey's equation[9]: $-\Delta F\,[\mathrm{Hz}] = 1.15\,\Delta m\,[\mathrm{ng}]$. The QCM was alternately immersed into aqueous solutions of PDDA (1 mg mL^{-1}) and DNA (1 mg mL^{-1}) for 10 min each immersion at 25 °C. A sample was taken, which was rinsed with pure water, dried with nitrogen gas, and then the frequency of the sample was measured. NaCl was added into both solutions at 0.1M, to increase amounts of the assembly.

Enzymatic degradation: The LbL assembly deposited on the QCM was immersed in DNase I Tris buffer solution with a concentration of 0.5 mg mL^{-1} in the presence of a suitable concentration of $MgCl_2$ and/or $CaCl_2$. After adequate reaction time, the QCM was rinsed with pure water, dried with nitrogen gas, and then the frequency was measured as above.

AFM: Images were obtained with a Digital Instruments NanoScope III operating in a tapping mode in air at ambient temperature. We did not perform any image processing other than flat leveling.

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