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Prolonged degradation of end-capped polyelectrolyte multilayer films

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Summary

Cationic chitosan (CT) and anionic dextran sulfate (Dex) were layer-by-layer (LbL) assembled from aqueous solutions containing 1 M NaCl on a quartz crystal microbalance (QCM) substrate, and the original films ((CT-Dex)$_3$-CT)) were end-capped with LbL assembly from CT solutions containing 1 M NaCl and Dex solutions without NaCl. The enzymatic degradation of films by chitosanase was quantitatively analyzed by QCM in terms of numbers of end-capping steps. The degradation of films end-capped with (Dex-CT)$_3$ was considerably prolonged when compared to those end-capped with other end-capping steps. A mechanism for the prolonged degradation was proposed by quantitative QCM data and zeta potential results.

Introduction

The layer-by-layer (LbL) assembly process provides the fabrication of polyelectrolyte multilayer films by alternate immersion of solid substrates into aqueous solutions of oppositely charged polyelectrolytes, resulting in the stepwise formation of polyion complexes on surfaces [1,2]. Electrostatic interactions and other universal interactions such as hydrogen bonds [3,4], charge transfers [5], van der Waals [6-8], and occasionally covalent bonds [9,10] can be utilized for LbL assembly. Since material surfaces are homogeneously coated with ultrathin films with regular nanostructures, potential applications of LbL assembled films to biomedical uses have been recently investigated. For instance, proteins adsorbed onto films were analyzed, and the amount and orientation of adsorbed proteins would change as dictated by the outermost layer component [11-16]. Secondary structures of adsorbed proteins were analyzed [17-19]. Furthermore, the interference or the acceleration of cellular adhesion [20-26] and pro versus anticoagulation of human whole blood [27,28] were analyzed in detail. Charged dyes were used as model drugs, conjugated into films, and released depending on changes in outer environments such as ionic strength and pH [29-33].

Another significant requirement for LbL assembled films for biomedical use is the controlled desorption of film components, for potentially releasing small molecules from films and material surfaces. The disappearance of hydrogen bonds by changing solution pHs [34,35] and of electrostatic interactions in highly ionic environments [4,36,37] resulted in the deconstruction of assembled films. Films composed of degradable polymers were also desorbed by spontaneous [38,39] or enzymatic [40-42] hydrolysis. In chitosan (CT) and dextran sulfate (Dex) assembly, films with Dex as outermost surfaces were preferentially hydrolyzed by cationic chitosanase due to the electrostatic condensation of enzymes on film surfaces [40]. This LbL change in hydrolytic rates was only observed when films were prepared in the presence of 1 M NaCl, because charge densities on film surfaces increase with increasing amounts of NaCl. Even with combinations of DNA and non-degradable synthetic polymers, films with synthetic polymer surfaces were also preferentially hydrolyzed [41]. The aforementioned observations strongly suggest that the charge density of film surfaces, which can be changed by the concentration of inorganic salts, is a key factor for the enzymatic degradation of LbL assembled films.

In this study, we demonstrated the enzymatic degradation of CT-Dex films composed of conventionally prepared inner films and end-capped thinner films. Assembly conditions for LbL assembly can be altered in a single assembly process, thereby showing changes in assembled structures. For inner original films, 1 M NaCl was added into both polymer solutions. On the other hand, for end-capped films, NaCl was only added into CT solutions. A prolonged degradation was expected to result from changing surface charge densities. The present multilayer end-caped film is schematically shown in Figure 1.

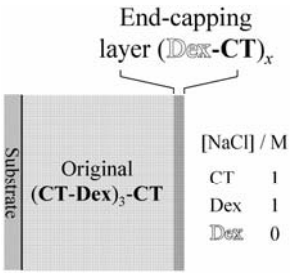

Figure 1. A schematic representation of the present multilayer end-capped films

Experimental

For the quantitative detection of LbL assembly and subsequent enzymatic degradation, a 9-MHz quartz crystal microbalance (QCM) substrate with an electrode diameter of 4.5 mm was used (USI system, Japan). Frequency shifts for the QCM (ΔF) were converted to assembled or desorbed polymer amounts (Δm), followed by Sauerbrey's equation: $-\Delta F$/ Hz = 1.15 x Δm/ ng [43]. Before assembly of CT and Dex, poly(diallyldimethylammonium chloride) (PDDA) (Aldrich) (M_w 100 000-200 000) and poly(sodium 4-styrenesulfonate) (PSS) (Aldrich) (M_w *ca.* 70 000) were assembled from aqueous solutions (1 mg ml^{-1}, 5 min immersion, 25 °C) for (PDDA-PSS)$_2$ with anionic surfaces. CT (M_w *ca.* 1 200 000) (Wako, Japan) with approximately 20 % chitin units and Dex (M_w *ca.* 1 400 000) (Wako) assembled on the substrate from

aqueous solutions of 25%-HCOOH (pH of solution=1) and water (pH of solution=6) (both polymer concentrations of 1 mg ml^{-1}), respectively, with 5 min immersions at 25 °C, following procedures outlined in previous studies [27,28,40]. After each immersion, films were rinsed with ultrapure water and dried with nitrogen gas. For original films lacking end-caps, $(CT\text{-}Dex)_3$-CT films were prepared in the presence of 1 M NaCl. Then, end-capped layers were assembled from a CT solution containing 1 M NaCl and a Dex solution (without NaCl) for a given number of steps. Film thickness was estimated by assuming a film density of 1 g cm^{-3}. Substrates coated with films were immersed into aqueous solutions of chitosanase (Wako) (M_w *ca.* 30 000, p*I* 9.3) (1 mg ml^{-1}, 0.1 M acetic acid buffer, pH 5.6, 37 °C). ΔF was measured in air after films were rinsed with ultrapure water and were dried with nitrogen gas. Zeta potentials of films were measured by ELS-8000 (Otsuka Electronics) in 0.1 M acetic acid buffer at 25 °C using glass slide substrates. The reported results were obtained from a minimum of three experiments.

Results and Discussion

Figure 2 shows a typical example of $-\Delta F$ against assembly step. Frequencies decreased with each step, thus indicating that the LbL assembly was performed successfully. Film thickness was also estimated by $-\Delta F$, assuming the film density to be 1.0 g cm^{-3}, following to our previous study [27,28]. In the presence of NaCl in both polymer solutions, film thickness increased exponentially. This observation may be caused by the penetration of polymers into films with ion pair rearrangements, and was similarly observed in other assembly systems [2]. Figure 3 shows the percent desorption of the original ($(CT\text{-}Dex)_3$-CT)), 6-step ($(CT\text{-}Dex)_3$-CT)-$(CT\text{-}Dex)_3$), and 10-step ($(CT\text{-}Dex)_3$-CT)-$(CT\text{-}Dex)_5$) end-capped films with all CT outermost surfaces against time, when films were immersed in chitosanase solutions. In all films, more than 90 % of CT and Dex were desorbed by enzymatic degradation after 80 min. Desorption did not simply occur, and could be divided into at least two processes. Plateau regions were observed after approximately 30 min degradation, and the remaining films reinitiated the degradation process. The degradation profiles did not correlate with assembly profiles of polymers. It is difficult to interpret the above-

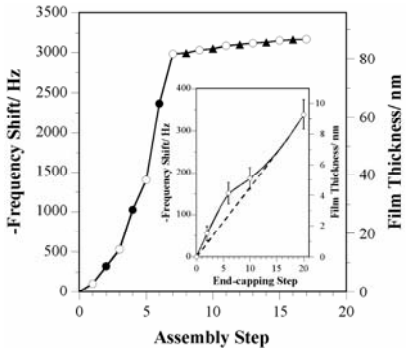

Figure 2. The frequency decrease against assembly step numbers. Open circles show CT assembly in the presence of 1 M NaCl. Closed circles and triangles show Dex assembly in the presence of 1 M NaCl and in the absence of NaCl. The inset shows a magnification of the end-capping process

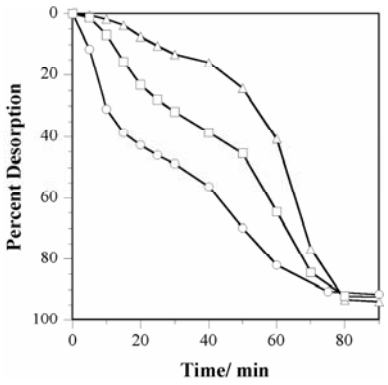

Figure 3. The percent desorption of LbL assembled films ((CT-Dex)$_3$-CT)-(CT-Dex)$_x$) against time. Circles, triangles, and squares show percent desorption of original (x = 0), 6-step (x = 3), and 10-step (x = 5) end-capped films

mentioned phenomenon. The rearrangement of polymers seemed to occur during the enzymatic reaction, resulting in a stepwise degradation of films. A similar phenomenon was not observed in our previous paper [40], since plots for time course analyses were smaller than those obtained in this study.

As expected, the end-capping of original film surfaces prolonged the desorption time, and is shown in Figure 3. A prolonged desorption time must be caused by changes in film surface structures, because the start of desorption seemed to be delayed. However, increases in end-capping steps did not result in further prolonged desorption times. In fact, films end-capped with 10 steps were more rapidly degraded than those with 6 steps. Times required for desorbing 14 nm thick from film surfaces (corresponding to increase in 500 Hz) are shown in Figure 4. Films with Dex and CT as outermost surfaces were examined. The degradation of films end-capped with 6 steps that had CT as outermost surfaces was clearly prolonged, suggesting that films end-capped with 6 steps have different assembly structures from those of other films. It is difficult to explain why films end-capped with 10 and 20 steps were more readily

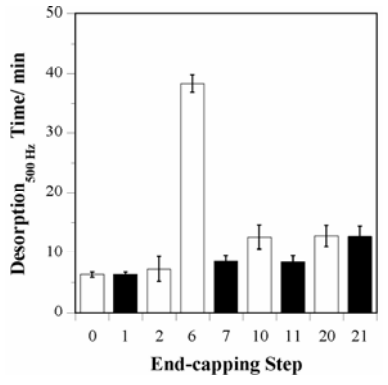

Figure 4. The desorption time for a 14 nm film thick against end-capping step numbers. Open and closed bars show desorption time for films with CT and Dex as outermost surfaces, respectively

degraded, even though enzymes will reach films within 6 steps during the degradation process. Once the degradation starts, a disordering of polymers in films, which might be correlated with rearrangements described above, might accelerate the degradation. As another possibility, film nanostructures may change with increasing numbers of end-capping steps.

On the other hand, the degradation of films with outermost Dex surfaces slightly increased with increases in end-capping steps. With the exceptation of films with 6-step end-capping, polymer components of outermost surfaces hardly affected degradation. Since NaCl was not added into Dex solutions and since assembly amounts of end-capping steps were much smaller than those in the presence of NaCl in both polymer solutions (see Figure 2), end-capping films seemed to be similarly assembled to a manner assembled in the absence of NaCl in both solutions (except for 6-step end-capped films). This must be the reason why outermost surfaces did not affect degradation, as previously reported [27,28]. In fact, films end-capped with 6 steps in the absence of NaCl in both polymer solutions did not show prolonged degradation, indicating that the present conditions are essential for the prolonged degradation of LbL assembled films (original data not shown).

It is essential to understand the unique degradation behavior of films end-capped with 6-step assembly processes. A quantitative analysis of end-capping processes is magnified in the inset of Figure 2. Greater amounts of polymers were assembled at around 6 steps, in which greater amounts of CT were assembled than those of Dex, suggesting that unusual assemblies had occurred. Zeta potentials of original, 6-step, and 10-step end-capped films are shown in Figure 5. Although all films have CT as outermost surfaces, original films showed negative zeta potentials. Since greater amounts of Dex were assembled than CT for original films (see Figure 2), Dex sulfate ions existing inside films possibly affect zeta potentials. It should be noted that all 2 or 3 numbers of sulfate ions linked to a single sugar unit must be unable to interact with a single CT amino group. On the other hand, end-capping changed zeta-potentials from negative to positive. Films end-capped with 10 steps had more positive zeta potentials than those with 6 steps. Even though greater amounts of CT might have been incorporated with 6 steps of end-capping, amino groups were not surface exposed. Until 6 steps, CT might compensate for sulfate ions remaining in original films. We have already reported that LbL assembled films are uniquely hydrolyzed [40]. Positively charged chitosanase in buffer solutions could electrostatically

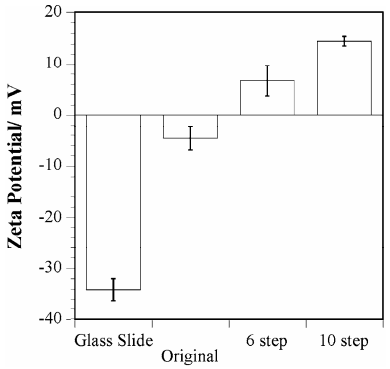

Figure 5. Zeta potentials of glass substrates, original, 6-step, and 10-step end-capped films

condense on negatively charged surfaces, and then hydrolyze chitosan near surfaces, resulting in film desorption. Furthermore, chitosanase can bind specifically to the chitosan substrate for hydrolysis. Therefore, enzymes can access film surfaces, and degrade chitosan. The access of chitosanase to surfaces with 6-step end-capped films seemed to be restricted due to moderate charge densities. However, the amount of chitosanase adsorbed onto films at 4 °C, a temperature at which chitosanase is inactive, was almost the same (adsorbed amounts were 0.39 μg cm^{-2}). Accordingly, it takes a longer time for adsorbed chitosanase to hydrolyze chitosan on films end-capped with 6 steps.

Conclusion

We propose herein a novel and simple method to prolong degradation times of LbL assembled films. We found that the end-capping thinner films prepared by adequate conditions, prolonged the degradation of assembled films. Quantitative QCM and zeta potential analyses suggested a mechanism for the prolonged degradation. The present study will contribute to the development of novel materials for biomedically-controlled release.

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