METHYL-π INTERACTIONS IN CATECHOL-CONJUGATED POLYMERS ENHANCES THICKNESS OF LAYER BY LAYER FILMS

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ABSTRACT

Layer-by-layer (LbL) assembly is a convenient and versatile surface functionalization method. However, the preparation of a thick film *via* LbL assembly is a challenging endeavor, as well as labor-intensive and time-consuming. In this study, we introduce a phenomenon called Self-Enhancement in the thickness of an LbL Film (SELF). We revealed that methyl-π interactions between the chemically tethered catechol (aromatic ring, π) and the acetyl (-CH_3) groups in a chitosan backbone occur in the SELF phenomenon. A thick free-standing film produced by SELF was shown to function as a high-capacity protein reservoir.

KEYWORDS: chitosan, methyl-π interaction, Layer by Layer (LbL)

INTRODUCTION

In the early 1990s, Decher and co-workers re-discovered the useful process of polymeric deposition on solid substrates, known as Layer by Layer (LbL) assembly, by depositing poly(styrene sulfonate) (PSS) and poly(allylamine hydrochloride) (PAH).[1] The LbL method is a facile technique with reproducible results. However in general, the deposition of several hundred layers has been required to obtain micrometer-thick films[14] Although the deposition of hundreds of layers is time-consuming and often requires automated equipment~~,~~ micrometer-thick films prepared by LbL assembly have advantages, including the high-capacity loading of molecules within the film, robust mechanical properties, the ability to tailor micro- and nanostructures, and the ability to integrate multiple functions into the thick film[14]. Therefore, fabrication of ultra-thick films with a thickness of several micrometers achieved by just a few depositions can open a way for practical uses in LbL assembly. In this study, we report that the deposition of only twenty bilayers of 0.1 wt% polymers results in an ultra-thick, free-standing film with a thickness of ~12 μm, which is unprecedentedly high compared to previous reports (Figure 1, red star). We found a novel inter-polymeric interaction mechanism called methyl-π, which plays a critical role in the dramatic increase in the film thickness. To our knowledge, this discovery of the presence of methyl-π interactions in a polymeric film is the first such report.

EXPERIMENTAL

We previously described a method to synthesize Chi-C.[2] The degree of catechol conjugation (DOC) of Chi-C was determined via ^{1}H-NMR spectroscopy (Bruker Avance, 400 MHz) by a method previously described.[2] Similarly, Hep-C was synthesized using an EDC reaction. For the LbL deposition of Chi-C/Hep-C, the substrates were coated overnight with Chi-C dissolved in Dulbecco's phosphate-buffered saline without magnesium and calcium buffer (1 mg/mL, pH 5.5) and placed on a 2D shaker at room temperature. The substrates were then washed with DDW and alternately coated in Hep-C dissolved DPBS buffer (1 mg/mL, pH 5.5) for 5 min and Chi-C solution for 5 min. The substrates were washed with DDW in between each coating step. To measure the dry thickness of the fabricated LbL coating, the LbL film deposited on substrates were partially exfoliated from the surface and measured with a Dektak-8 surface profilometer (Veeco, Plainview, NY, USA). The size of the image was set to 500 by 500 μm. The thickness was measured four times at randomly selected locations. The Chi-C/Hep-C film was also evaluated with a BIACORE 3000 (GE Healthcare, Little Chalfont, Buckinghamshire, UK).

978-0-9798064-8-3/μTAS 2015/$20©15CBMS-0001

RESULTS AND DISCUSSION

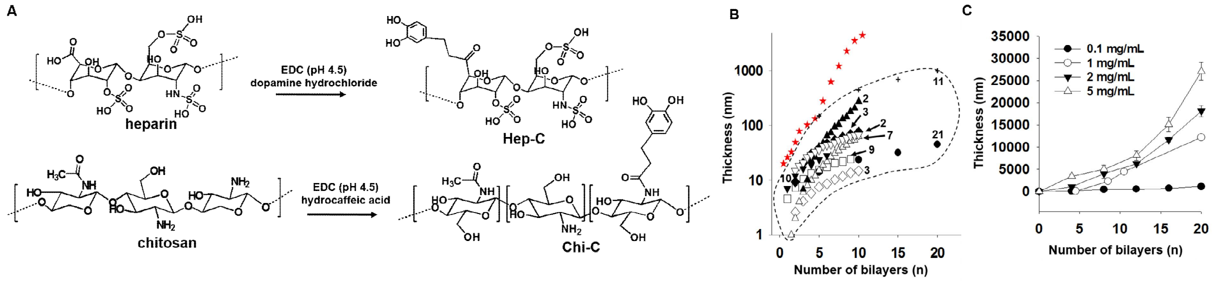

Figure 1. (A) Chemical structures of Chi-C and Hep-C. Thickness evolution of Chi-C/Hep-C (red stars, 1 mg/mL) and comparison with previous studies on logarithmic (B) and linear (C) scales: HA/COL[12] (open star), chitosan/HA[8] (■), bPEI-C/PAA-C[11] (┼), polyester dendrimer[9] (□), polyaniline/PAAm[3] (◆), polyaniline/SPS[3] (◇), PEI-C/HA-C[21] (●), chitosan/heparin[7] (△), PGA/PLL[2] (▲), PSS/PAH[2] (▽), PAA/PVP[10] (▼) and Chi-C/Hep-C (red star). Chi-C/Hep-C (red star) was measured using a surface profilometer. (B) The LbL films with thicknesses of less than 1 μm at 20 bilayers are marked with a black dashed line. (C) The Chi-C/Hep-C fabricated at different polymer concentrations.

Chi-C and Hep-C (Figure 1A) were synthesized as shown. Chi-C/Hep-C (0.1 wt%) LbL assembly reached an average thickness of up to 570 nm (dry thickness) per layer in the exponential growth region, (Chi-C/Hep-C)$_{16-24}$, (Figure 1B, red star). The thickness of Chi-C/Hep-C also increased according to concentration of the polymers (Figure 1C). Chi-C/Hep-C at 2 and 5mg/mL(0.2 and 0.5 wt%, respectively) further enhanced thickness of Chi-C/Hep-C (0.1 wt%). In polyelectrolyte LbL assembly, finite growth in the layer thickness caused by charge repulsion is a general phenomenon. Charged polymer molecules in solution are not deposited when the surface charge is the same polarity.

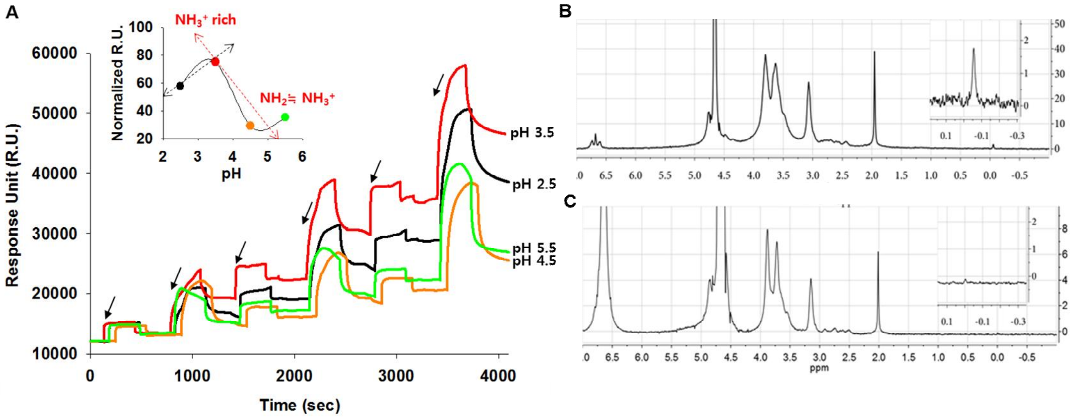

Figure 2 (A) R.U. profile of (Chi-C/Hep-C)$_3$ with Chi-C at pH 2.5, 3.5, 4.5, and 5.5. The total change in baseline is plotted in the inset. The arrows indicate alternating injections of Chi-C followed by Hep-C solutions. (B) ^{1}H-NMR spectra of Chi-C in D_2O and (C) Chi-C in 1 M deuterated guanidine chloride. The zoom-in insets emphasize the proton peaks in the negative ppm region disappearing when guanidine is added.

Therefore, we hypothesized that additional catechol-mediated interactions might be actively involved. Theoretically, the catechol groups can participate in various interactions, such as 1) Schiff base formation (irreversible covalent), 2) π-π stacking, 3) cation-π, and 4) methyl-π interactions (reversible). We explored the possibility of a cation-π interaction. The population of cations from the amine groups of chitosan chains was increased as acidity increased (from pH 5.5 to 2.5, red dashed line in Figure 2A) and therefore the film thickness was expected to increase. However, we found that the thickness of (Chi-

C/Hep-C)$_3$ decreased from pH 3.5 to pH 2.5 when amine groups are more cationic. This is due to increase in Chi-C charge density at pH 2.5 which prevents inter-diffusion of Chi-C polymers and thus decreases thickness. The two arrows, red dashed line (effect on thickness due to cation-π interaction) and blue dashed line (effect on thickness due to charge density) counteracts on each other (Figure 2A) and thus the data shows that cation- π as shown from red dashed line is not a major player at pH 5.5.

The acetyl group in chitosan presents a methyl moiety (-CH$_3$), and the catechol group has π electrons. In general, when a methyl proton closely interacts with the π electrons in benzene group-containing amino acids, such as phenylalanine, tyrosine, or tryptophan, a significant electron-rich environment is created. This special situation occurs at a core environment of proteins. The high level of electron shielding thus results in the appearance of a ^{1}H-NMR peak in the negative ppm region.[21] We previously reported that chitosan exhibits a folded structure when catechol groups are conjugated[24]. Therefore, we performed a ^{1}H-NMR analysis of Chi-C and found a distinct proton peak at -0.025 ppm (Figure 2B). We assigned the negative peak as the methyl proton closely interacting with the π-electrons in catechols. When we added 1 M deuterated guanidine chloride (which can disrupt methyl-π interactions) the negative ppm peak was completely removed (Figure 2C).

CONCLUSION

In this study, we reported that a new interaction mechanism, known as methyl-π, can occur in the LbL assembly of catechol-conjugated polymers. The –CH$_3$ in chitosan and the π electrons in catechols are involved in the film formation, which results in unprecedentedly thick, free-standing film formation. The phenomenon of self-enhancement of the film thickness originates from the additional methyl-π interaction, which can partially overcome the charge repulsion between the assembled polyelectrolytes.

ACKNOWLEDGEMENTS

This study was gratefully supported by the grants of Mid-career Scientist Award from the National Research Foundation of S. Korea (2014002855). Also, this study was supported by World Premier Material Development Program from the Ministry of Knowledge and Economy (10037915).

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