Protein Adsorption is Dependent on Substrate Polymer Polymorphs

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Adhesion force, quartz crystal microbalance (QCM), and attenuated total reflection infrared (ATR-IR) measurements revealed that native proteins weakly adsorbed onto poly(lactide) (PLA) stereocomplexes with 3_1 helical (β-form) crystals when compared with poly(L-lactide) (PLLA) homogeneous crystals with 10_3 helical (α-form) crystals. These observations suggest that substrate polymer polymorphs can be significant factors that determine protein-adsorptive properties.

The physical adsorption of proteins onto material surfaces is an initial key process that determines material potentials in biomedical fields.[1] The fully covered monolayer adsorption of native proteins is a significant requirement for certain applications. Protein adsorption is generally dependent on substrate properties such as hydrophobicity and charge.[1] However, other essential parameters for protein adsorption are also important. In this study, we show for the first time in the literature that protein adsorption is strongly dependent on polymorphs of substrate polymers.

To demonstrate the aforementioned novel concept, crystalline PLA substrates which have attracted a great deal of attention in biomedical fields were selected.[2] Optically active poly(L-lactide) (PLLA) and poly(D-lactide) (PDLA) form respective left- and right-handled 10_3 helical (α-form) crystals in homogeneous solids.[3] On the other hand, stereocomplexes formed between PLLA and PDLA are composed of respectively handled 3_1 helical (β-form) racemic crystals.[3] Slight differences in the aforementioned crystal structures makes these compounds possible candidates to demonstrate protein adsorption dependent on substrate polymorphs.

PLLA ($M_n = 635900$, $M_w/M_n = 1.3$) and PDLA ($M_n = 542600$, $M_w/M_n = 1.5$) were kindly provided as a gift by PURAC Biochem (Netherlands). Fourteen layer-by-layer assembly steps between PLLA and PDLA were used to prepare 11 nm-thick stereocomplex films containing 90.7% of the β-form.[4] Conventional spin-coating and subsequent thermal treatments were used to prepare 81 nm-thick PLLA films containing 83.5% of the α-form.[4] As a model protein, human serum albumin (HSA, 66 kD) was used for all analyses. Experimental details are summarized in the Supporting Information.[9]

A typical force-displacement curve obtained from combining the HSA-modified cantilever tip and the stereocomplex surface is shown in Figure 1a. When the tip approached the surface, steric repulsion was observed at certain displacements. During the subsequent retraction, adhesion forces between the tip and the surface were observed successfully. Histograms of the occurrence of the adhesion forces for the complex and PLLA surfaces are shown in Figure 1b. It is clear that the adhesion force against

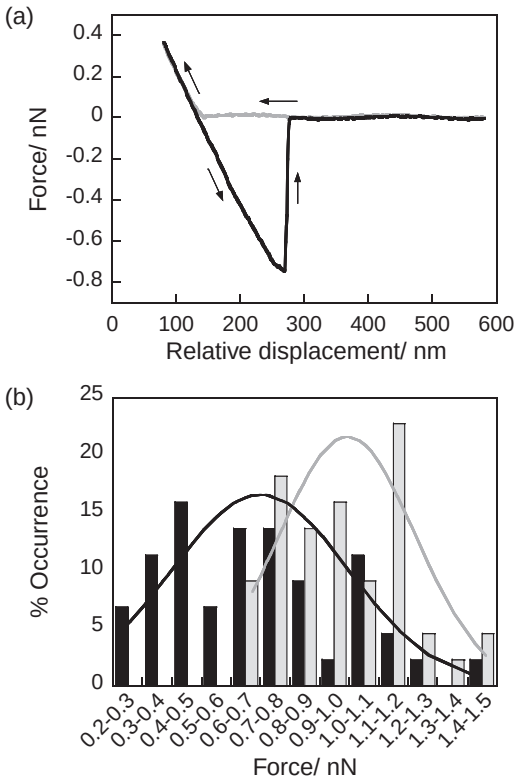

Figure 1. (a) A representative force-displacement curve obtained with the HSA-modified cantilever for the PLA stereocomplex film. (b) Histograms of maximum attractive forces with Gaussian distributions, shown in black for stereocomplexes and gray for homogeneous films, respectively.

the complex is smaller than that against the PLLA. Mean values $\pm$ standard deviations were estimated to be 0.68 ± 0.30 and 0.97 ± 0.21 nN for the complex and PLLA surfaces, respectively, thereby indicating that HSA weakly adhered onto the β-form crystal in comparison to the α-form. It is noted that measurements were performed with the same tip for both the complex and PLLA surfaces. Therefore, we can ignore the difference in amounts and orientation of immobilized HSA on the cantilever. Both surfaces contained amorphous regions; however, those contents are small. Even though the amorphous regions influence the adhesion force, it must be very few. Accordingly, differences in adhesion forces dependent on the polymorph are reliable.

To obtain further information on protein adsorption dependent on PLA polymorphs, apparent adsorption constants (K_{app}s) of HSA in equilibrium states were determined from Langmuir adsorption isotherms (Figure 2a) using the QCM substrate.

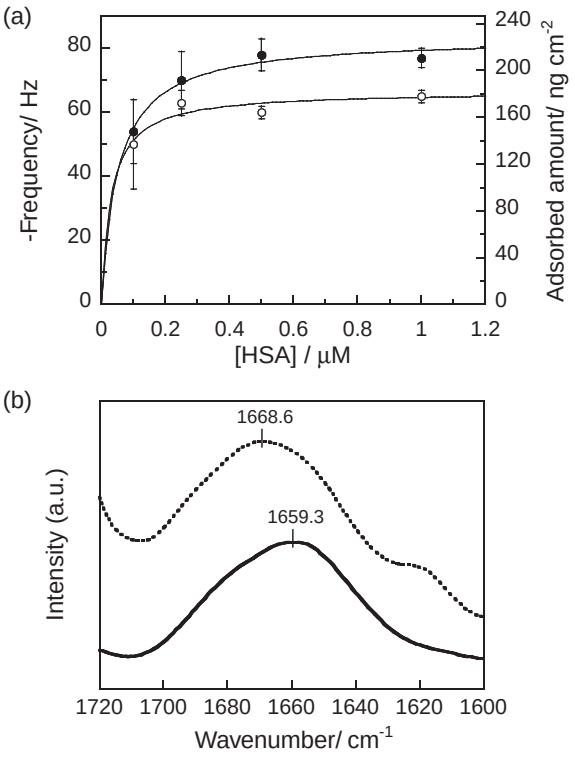

Figure 2. (a) Adsorption isotherms for HSA on the stereocomplex (closed circles) and the PLLA (open circles) film surfaces. (b) Typical ATR spectra of HSA adsorbed on the stereocomplex (solid line) and the PLLA (dotted line) film surfaces.

The K_{app} value of $1.9 \times 10^7\,M^{-1}$ for the complex surface was slightly smaller than that of $3.3 \times 10^7\,M^{-1}$ for PLLA. These observations indicate that proteins weakly adsorbed onto the β-form crystal, supporting the aforementioned adhesion force difference. On the other hand, stronger interactions might enhance protein denaturation after adsorption, resulting in an increase in the contact area per a single protein molecule. Therefore, adsorbed amounts to the α-form seemed to be decreased when compared to the β-form.

An ATR-IR study suggested that the denaturation of adsorbed HSA was significantly suppressed on the complex surface (Figure 2b). The amide I band range from 1600 to $1700\,cm^{-1}$ is sensitive to a peptide's secondary structure, where α-helices, β-sheets, β-turns, and extended coil structures absorb at different frequencies.[5] It is known that the amide I band shifts to higher wavenumber with increasing random-coil contents.[6] The peak frequency of adsorbed HSA on the complex was observed at $1661.1 \pm 1.6\,cm^{-1}$, while that on the PLLA shifted to higher wavenumber ($1667.6 \pm 1.1\,cm^{-1}$). The aforementioned observations suggest that protein unfolding on the complex was suppressed by the soft-landing of proteins, which could be explained by the smaller adhesion force and the smaller K_{app}. We did not compare the spectra of powder HSA with those of adsorbed HSA, since it is known that amide peak positions shift due to different hydration states.[7]

Since the adhesion force is composed of the sum of individual interactions such as H-bonding, van der Waals forces, and hydrophobic effects between proteins and non-charged PLAs, fewer contacts result in smaller adhesion forces. One possible explanation for the smaller adhesion force between the protein and the PLA β-form is the fewer number of H-bonds. In the PLA β-form, weak H-bonding such as specific $CH_3\cdots O{=}C$ and $C_\alpha H\cdots O{=}C$ interactions between both stereoisomers of PLAs were formed.[8] Therefore, both the number of H-donors and the number of H-acceptors of the PLA β-form, which are able to contribute proteins to H-bonds, are decreased when compared to the PLA α-form, resulting in the weak adhesion of proteins. On the other hand, surplus H-bonds formed between proteins and PLA α-forms may lead to the unfolding of proteins after adhesion. There is not a large difference in the contribution of van der Waals forces of proteins against PLA surfaces based on structural differences between 10_3 helices (α-form) and 3_1 helices (β-form) of PLA, because another protein, carbonic anhydrase, that has a different tertiary structure from HSA, also shows similar adsorption behaviors.

Herein, for the first time in the literature, we revealed that protein adsorption was dependent on the polymorphism of the polymer using structurally well-defined surfaces composed of PLA stereocomplexes and homogeneous PLLA. Proteins weakly adsorbed onto β-form stereocomplexes, and protein unfolding following adsorption were suppressed on the stereocomplex. The present observations demonstrate the general importance of polymeric polymorphs towards adsorbed proteins as well as the biomedical characteristics of PLA surfaces. The detailed mechanism and the activities of other functional proteins adsorbed on PLA surfaces will be reported in future manuscripts.

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9 Supporting Information is available electronically on the CSJ-Journal web site; http://www.csj.jp/journals/chem-lett/.

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