Quantification of the Influence of Protein-Protein Interactions on Adsorbed Protein Structure and Bioactivity

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Statement of Purpose

To control the bioactive state of a protein in its adsorbed state, it is important to understand not only the role of protein-surface interactions (PSI) but also the influence of protein-protein interactions (PPI) on the structure of adsorbed proteins. The objective of the current study was to quantify the structure and the corresponding bioactivity responses of adsorbed proteins on different surfaces when the influence of PPI effects were varied by controlling the bulk solution concentration of the protein, adsorption time in protein solution, and equilibration time following adsorption in pure buffer.

Materials and Methods

Protein and adsorbent surface models: Hen egg white lysozyme (HEWL) dissolved in 10 mM potassium phosphate buffer (PPB, pH 7.4) was adsorbed on fused silica glass (GLASS), high density polyethylene (HDPE), poly(methyl-methacrylate) (PMMA).

Protein Adsorption: Proteins were adsorbed onto each adsorbent material from a series of protein concentrations (0.03 mg/mL – 1 mg/ml) for 2 hours to saturate the surface, after which surfaces were rinsed under running water and incubated at room temperature for 24 hours in pure PPB to equilibrate the adsorbed proteins.

Circular Dichroism (CD) Spectroscopy: The structure of HEWL in solution, the amount of protein adsorbed on each surface, and the subsequent adsorption-induced conformational changes of the proteins due to adsorption on various material surfaces were determined using CD spectropolarimetry. The solution structure of the proteins was determined in quartz cuvettes (Starna Cells) while the structure of the adsorbed proteins was determined using a custom-designed cuvette.[1]

Bioactivity Assays: Turbidometric assays to monitor the enzymatic activity of HEWL were carried out in the same custom-designed cuvettes.[1] Bioactive substrates were prepared in PPB to a final concentration of 60 mg/L *Micrococcus lysodeikticus* (Sigma M3770) and the assays to determine the enzymatic bioactivity were done at pH 7.4 for a time period of 10 min at 450 nm.

Results and Discussions

A new experimental approach to quantitatively study the influence of PPI effects on the conformation and bioactivity of adsorbed protein has been developed by varying the adsorption time, equilibration time following adsorption, and protein bulk concentration. This technique enables us to accurately track the structural changes of adsorbed proteins by CD under varied PPI conditions and assess their influence on adsorbed-state bioactivity.

The results shown in Figure 1 indicate that when adsorbed under low concentration (i.e., minimal PPI effects), adsorption on GLASS induced almost full loss of helical structure, HDPE induced moderate loss, while PMMA minimally influenced the degree of helicity. Interestingly, as PPI effects were increased by adsorbing under higher solution concentration, the influence of PPI effects tended to stabilize the protein against unfolding on GLASS, while having a mild destabilizing effect on HDPE and a strong destabilizing effect on PMMA. The corresponding bioactivity results (Figure 2) of adsorbed HEWL on the GLASS and HDPE surfaces suggest the general trend that the bioactivity losses following adsorption were due to the conformational changes of adsorbed HEWL, while additional factors evidently played a substantial role on PMMA.

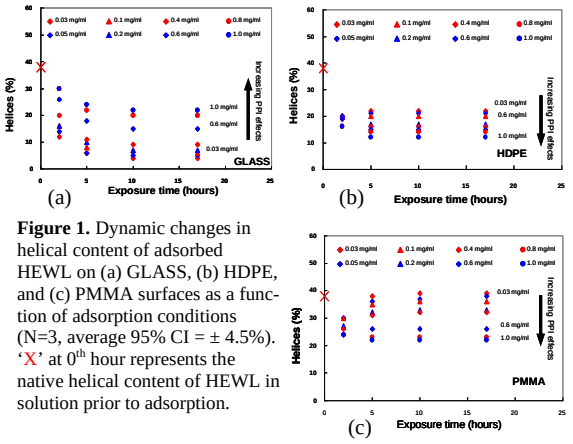

Figure 1. Dynamic changes in helical content of adsorbed HEWL on (a) GLASS, (b) HDPE, and (c) PMMA surfaces as a function of adsorption conditions (N=3, average 95% CI = ± 4.5%). 'X' at 0th hour represents the native helical content of HEWL in solution prior to adsorption.

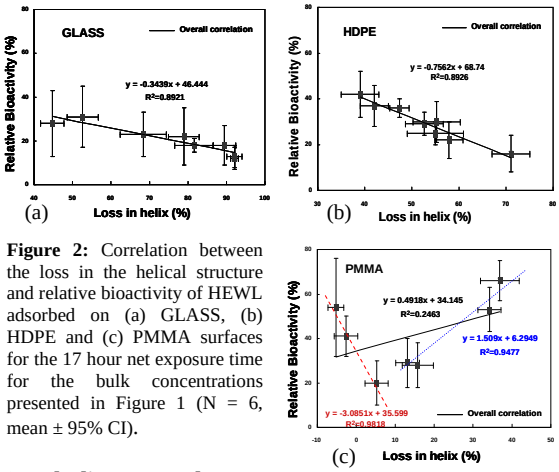

Figure 2: Correlation between the loss in the helical structure and relative bioactivity of HEWL adsorbed on (a) GLASS, (b) HDPE and (c) PMMA surfaces for the 17 hour net exposure time for the bulk concentrations presented in Figure 1 (N = 6, mean ± 95% CI).

Concluding Remarks

In this study, we present the first application of a method to characterize the adsorption response of HEWL on GLASS, HDPE, and PMMA surfaces when adsorbed under varying influences of PPI effects. These results provide fundamental insights into the molecular mechanisms mediating the adsorption processes, and show that PPI effects can influence both the structure and the bioactivity of an adsorbed protein in distinctly different ways depending on the characteristics of the adsorbing surface.

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References: 1. Sivaraman, B.; Fears, K. P.; Latour, R. A., *Langmuir* **2009,** 25, (5), 3050-3056