Determination of Orientation and Adsorption-Induced Changes in Tertiary Structure of Proteins on Material Surfaces by Chemical Modification and Peptide Mapping

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Statement of Purpose: The chemical modification of targeted amino acid residues followed by peptide mapping via mass spectrometry (AAL/MS) is a promising technique to provide detailed information on the orientation and structure of adsorbed proteins. However, the potential of this method is largely undeveloped at this time. The objective of this research was therefore to extend these capabilities by developing and applying chemical modification and peptide mapping techniques for a range of amino acid types to identify the dominant configurations of an adsorbed protein on a material surface.

Methods: The adsorption responses of hen egg white lysozyme (HEWL) and bovine pancreatic ribonuclease-A (RNAse-A) on fused silica glass (glass), high-density polyethylene (HDPE) and poly(methyl methacrylate) (PMMA) was investigated in potassium phosphate buffer (PPB, pH 7.4). The proteins were adsorbed onto each adsorbent material from 0.03 mg/mL and 1.0 mg/mL protein solution concentrations for 2 hours, after which surfaces were diluted under running nanopure water and incubated at room temperature for at least 3 hours in pure PPB to equilibrate the adsorbed proteins [1]. Experimental data on the adsorbed configuration of the protein on each surface was subsequently mapped by combining the labeling profiles obtained from chemical labels covalently linked to the side chains of multiple amino acids that were independently applied, following which the location of the labeled amino acids was determined by MS. In this study, five amino acids (Arg, Lys, Asp, Glu, and, Trp) in HEWL and six amino acids (Arg, Lys, Asp, Glu, His, and Tyr) in RNAse-A were targeted. For each of the proteins, in order to combine the results from different labeling processes, the intensity of a unique peptide segment without the target amino acids was used as the internal standard to normalize the intensity shifts from different labeling processes to an equivalent level. Accordingly, the profile values for the combined set of targeted amino acids for HEWL and RNAse-A were mapped onto the respective native tertiary structures (1GXV for HEWL and 6RSA for RNAse-A), which were obtained from the Protein Data Bank and visualized with UCSF Chimera. [2-4]

Results: Figure 1 shows the labeling profile of targeted residues in HEWL on each of the different surfaces when adsorbed from 0.03 mg/mL and 1.00 mg/mL protein solution concentrations, indicating different adsorption behavior for each condition. The data presented in Figure 1 was then mapped onto HEWL's tertiary structure (Figure 2) to represent HEWL adsorbed from 0.03 mg/ml and 1.00 mg/mL solution concentrations onto the glass surface. Figure 2 shows that the amino acid solvent accessibility is distinctly different for HEWL adsorbed from the different solution conditions. Under 0.03 mg/ml solution conditions, HEWL adsorbed predominantly along one patch of positively charged amino acid residues (orange and yellow) that are positioned both at one end and along one side of the protein. When adsorbed from 1.00 mg/ml solution concentration, the profile shifts towards being more concentrated along the sides of the protein but with a lesser degree of loss of solvent accessibility, presumably due to protein-protein effects interfering with the ability of the HEWL to be oriented by the electrostatic attraction by the surface.

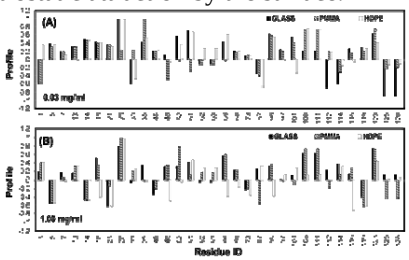

Figure 1. Labeling profile of HEWL on Glass, PMMA, and HDPE surfaces when adsorbed under (A) 0.03 mg/mL and (B) 1.00 mg/mL protein solution concentrations. Profiles within about ± 0.1 of 0.0 can be considered to be not significantly different from the solution state (n = 3, mean ± 95% C.I).

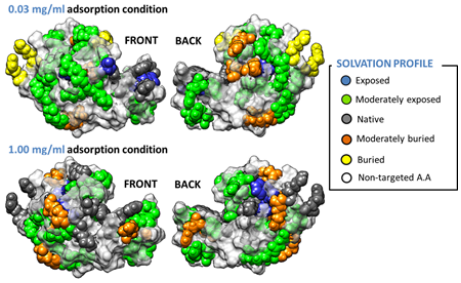

Figure 2. Solvation profiles of residues in HEWL adsorbed on glass from 0.03 mg/mL and 1.00 mg/mL protein solution concentration.

Conclusions: This study quantitatively demonstrates that adsorption-induced effects on the structure of proteins at the residue level depend on the type of surface to which it is adsorbed and the solution concentration that it is adsorbed from. Of particular importance, the developed technique allows the labeling profiles from multiple amino acids within an adsorbed protein to be combined together by normalizing their profiles to a common basis. This capability provides the potential to further expand this approach to a broader set of target amino acids so as to obtain additional detailed information to probe the structure of adsorbed protein layers on surfaces to further our understanding of protein adsorption behavior.

References: [1] Wei Y, et al. Colloids and Surfaces B-Biointerfaces. 2013;110:363-71. [2] Refaee M, et al. Journal of Molecular Biology. 2003;327:857-65. [3] Pettersen EF, et al. J Comput Chem. 2004;25:1605-12. [4] Berman HM, et al. Nucleic Acids Res. 2000;28:235-42.

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