pH Dependence of Albumin Spatial Distribution on a Patterned PS/PMMA Surface

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Introduction: Detecting and understanding protein adsorption on surfaces that are patterned at the nanometer-scale can help understand the mechanism of protein adsorption and contribute to biomaterial development. Spatial control of protein adsorption on a patterned surface is especially important in biosensors. Recently we have shown that synchrotron based soft X-ray photoemission electron microscopy (X-PEEM) can simultaneously map adsorbed protein and an underlying polymer substrate at high spatial resolution (~70 nm), thus determining adsorption site preferences. Using X-PEEM, we studied albumin adsorption at sub-monolayer levels on a phase segregated polystyrene/ polymethylmethacrylate (PS/PMMA) blend surface under different adsorption conditions. The PS/PMMA interfaces were found to be the first adsorption sites of albumin adsorption from unbuffered aqueous solutions over a range of concentrations [1]. A redistribution of adsorbed albumin after longer (60 min) exposure times was observed, possibly due to surface diffusion. Here we report results of an X-PEEM study of the adsorption of 0.01 mg/ml human serum albumin (HSA) on a PS/PMMA blend at five different pH values (2.0, 4.0, 7.2, 8.6 and 10.0) in order to investigate the influence of albumin conformation on adsorption site preferences.

Materials/Methods: The substrate was a ~40 nm thick, spun-cast PS/PMMA blend thin film on a native oxide Si wafer. PS (MW = 1.07 M) and PMMA (MW =312 K) from Polymer Source Inc were used. A 30:70 w/w PS/PMMA (1% by weight) toluene solution was spun cast (4000 rpm, 40 s) and annealed at 160°C for 12 h. Solutions of HSA (0.01 mg/ml) at different pH were prepared by successive dilution from 0.1 mg/ml stock and the pH values were set by dropwise addition of HCl or NaOH. The protein exposure was performed by placing a PS/PMMA/Si substrate into a well of a Fisher multiwell plate containing the albumin solution. After 20 min, the exposed substrate was transferred to pure solvent in another well to dilute the protein. Four 2 minute cycles ensured that all solution protein was removed prior to drying.

The X-PEEM at the Advanced Light Source bending magnet beamline 7.3.1 was used. The light was elliptically polarized with 70-80% right circularly polarized light. C1s image sequences were recorded and analyzed to generate quantitative thickness maps of the PS, PMMA and albumin components using methods outlined in [1].

Results/Discussion: As the pH changes from 2 to 10, albumin undergoes five reversible conformation changes [2] which involve unfolding [3] and folding [4]. **Fig. 1** summarizes the results of the X-PEEM study in terms of Venn diagrams where the total areas are proportional to the total amount (in terms of mean thickness [1]) of adsorbed albumin, and the pie areas are proportional to the amounts adsorbed on the PS, PMMA and interdomainal interface regions. The PS/PMMA interface was the preferred adsorption site at all values of pH studied. The proportions adsorbed on the PS and PMMA regions were similar at pH values of 7.2, 2 and 10.0. However a completely different situation was observed at pH=4, where PMMA was strongly preferred, and at pH=8.6 where PS was strongly preferred.

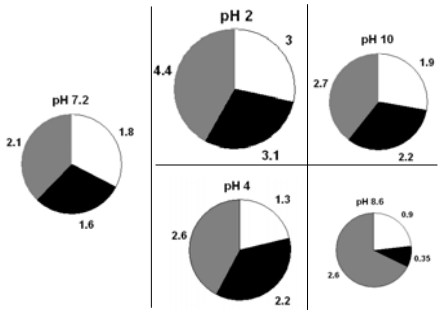

Fig. 1 Albumin adsorption on different regions (white—PS, black—PMMA, grey—interface) as a function of pH. The numbers are mean thickness (nm) on each region.

The change in site preference with pH can be explained in terms of changes in the surface properties of albumin in the different conformations. At neutral pH, the albumin surface has similar amounts of hydrophobic and hydrophilic residues and shows similar adsorption to the PS and PMMA regions. At pH 4, albumin unfolds, exposing more hydrophilic residues which favors adsorption on the PMMA. At pH 8.6, albumin contracts by increased internal hydrogen bonding. Adsorption is then more extensive on the PS than on the PMMA domains, possibly because there are relatively more hydrophobic residues at the albumin surface.

Conclusions: C1s X-PEEM was used to measure amounts and spatial distributions of albumin adsorbed on a patterned PS/PMMA surface at different pH. The changing distributions were rationalized in terms of known conformation changes with pH [2-4].

References:

[1] Li, L et al. *J. Phys Chem B*. 2006; 110: 16763-16773.
[2] Foster, J.F. *Albumin Structure, Function and Uses*, Pergamon, Oxford, 1977, pp.53-84.
[3] Carter, D. C. *Adv Protein Chem*. 1994; 45: 153-203.
[4] Qiu, W. *J Phys Chem B*. 2006; 110: 10540-10549.

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