Time-Dependent Conformational Changes in Adsorbed Albumin and its Effect on Platelet Adhesion

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Statement of Purpose: Albumin (Alb) is one of the first proteins to adsorb on biomaterial surfaces and it is minimally desorbed by other proteins (e.g., Fg, IgG), thus indicating that it is typically a major component of the protein layer on a biomaterial upon blood contact. Recent studies by our group using surface chemistry and solution concentration to influence the degree of adsorption-induced Alb unfolding showed that platelets can actually adhere to adsorbed Alb, but only if it undergoes more than a critical degree of adsorption-induced unfolding. We also found that it was unlikely for Alb to unfold to this critical degree when adsorbed under physiological conditions.[1] This raised the question of whether Alb initially adsorbed in its near-native state would undergo further unfolding with time; and if so, whether this would then induce platelets adhesion. This question is clinically relevant as late stent thrombosis has been correlated with prolonged exposure of stent surfaces to blood. To our knowledge, this is the first study to examine long-term, time-dependent conformational changes of an adsorbed plasma protein and its effect on platelet adhesion.

Methods: Self-assembled monolayers (SAMs) of alkanethiols with CH_3- and OH-terminal functionalities (Sigma-Aldrich) were formed on gold-coated quartz slides (CD studies) or cover-glasses (platelet adhesion studies). Alb (Sigma-Aldrich) was preadsorbed on the SAM surfaces at 10.0 mg/mL bulk solution concentration, and the percentage α-helix and β-sheet of adsorbed Alb was deconvoluted using the CDPro software[2] from the CD spectra obtained using a Jasco J-810 spectropolarimeter.[3] Alb surface coverage was calculated based on the absorbance of the sample resulting from the peptide peak at 195 nm.[3] Adhesion of washed platelets to these SAMs was performed and quantified using a lactate dehydrogenase (LDH) assay. The Alb-coated SAMs were incubated in fresh buffer containing 0.1% antibiotic-antimycotic (Mediatech) and incubated at 37ºC for up to 6 months, with this buffer replenished every 2 weeks. CD and platelet studies were carried out on CH_3 and OH SAMs incubated under these conditions at 3 & 6 month time-points post-Alb adsorption.

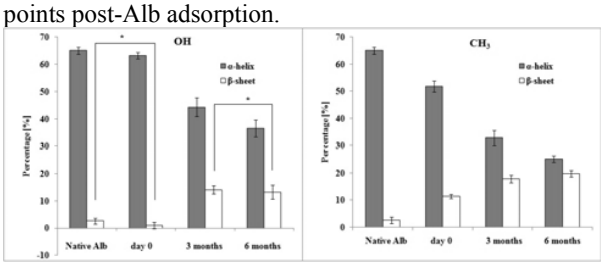

Figure 1. Secondary structural changes in Alb adsorbed on OH (left) and CH_3 (right) SAMs, from 10.0 mg/mL bulk solution concentration for time points of 0, 3, and 6 months (n=10, mean ± 95% CI). * denotes that mean values are not shown to be statistically different, $p > 0.05$.

Results: The adsorbed Alb underwent a distinct loss in α-helix with increasing residence time over 6 months, and the hydrophobic CH_3 SAMs induced greater unfolding compared to the hydrophilic OH SAMs, as shown in Fig.1. The surface coverage of adsorbed Alb remained consistent over this duration (Table 1), indicating that the protein layer did not desorb during this time period.

Table 1. Amounts of Alb adsorbed on CH_3 and OH SAMs from 10.0 mg/mL bulk solution concentrations, for 0, 3, and 6 month residence times. (n= 6, mean ± 95% CI).

Surface	Day 0 (μg/cm²)	3 months (μg/cm²)	6 months (μg/cm²)
CH_3	2.65 ± 0.24	2.63 ± 0.30	2.83 ± 0.18
OH	1.63 ± 0.23	1.64 ± 0.19	1.73 ± 0.16

Platelet adhesion to both SAMs was minimal at day 0, but significantly increased at 3 and 6 months, with the CH_3 SAM exhibiting higher platelet adhesion compared to the OH SAM at both time points, as shown in Fig 2. The net trend followed the relationship indicated in our prior studies,[1] with increased platelet adhesion to Alb attributed to aging-induced unfolding. These results conclusively illustrate that an irreversibly adsorbed Alb layer undergoes aging-induced unfolding over time, and this can lead to platelet adhesion in a manner very similar to adsorption-induced Alb unfolding caused by system conditions without aging.

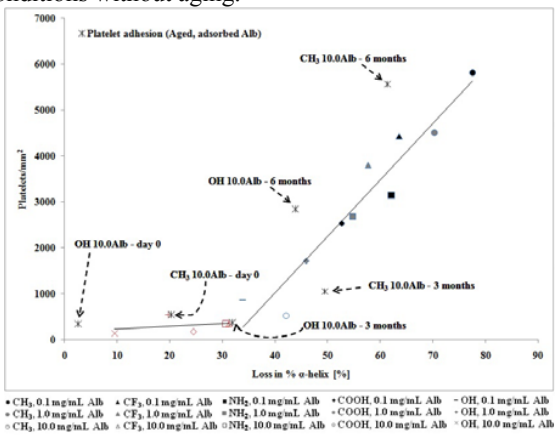

Fig 2. Platelet adhesion to adsorbed Alb aged on the OH and CH_3 SAM surfaces for 0, 3, and 6 months, as a function of the degree of unfolding, as measured by the percentage loss in α-helix. These data points are overlaid for purposes of comparison on the previous results obtained by our group for results obtained without aging.

Conclusions: We conclude that the relationship between platelet adhesion and protein adsorption is more complex than previously understood. We suggest that rather than preventing protein adsorption, the biomaterials field should focus on adsorbing proteins (*i*) such that their native structure is minimally perturbed, and (*ii*) in a reversible manner such that the adsorbed protein does not undego aging-induced unfolding on the surface. Further studies with other plasma proteins are necessary.

References: 1) Sivaraman et al., Biomaterials 2010, 31: 1036. 2. Sreerama et al., Anal. Biochem. 2000, 287:252. 3. Sivaraman et al., Langmuir 2009, 25: 3050.