Apolipoprotein AI, a Major Player in Blood Material Interactions.

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Introduction: Protein adsorption is widely recognized as the first event when a material contacts tissue. A significant amount of work has been done on elucidating the "proteome of protein adsorption", i.e. identifying and determining the amounts of the myriad proteins which adsorb to biomaterial surfaces. In recent work we identified apolipoprotein AI (ApoAI) as a major protein bound to a wide variety of surfaces following plasma or blood exposure both in vitro and in vivo.[1] However, the general significance of this phenomenon remains undetermined.

ApoAI is present in plasma at the relatively low concentration of ~1.2 mg/mL, 90-95% of which is bound to high density lipoprotein (HDL) particles, and 5-10% circulates as free, or unbound, ApoAI. It is not clear whether the apoAI adsorbed to biomaterials is HDL-bound or free. We previously identified ApoAI on biomaterial surfaces qualitatively using SDS-PAGE, Western blotting, 2D gel electrophoresis, and mass spectrometry. The work reported here aims to quantify the amounts ApoAI bound to biomaterial surfaces using radiolabeling methods. Data on Tecothane, a commercial segmented polyurethane are reported.

Materials and Methods: Tecothane film was exposed to human plasma (1 min to 24 h), rinsed with buffer, and the protein eluted by incubation with 2% SDS. SDS-PAGE (12% acrylamide) was carried out at 200V for 45 minutes, the protein transferred to PVDF membranes, and stained with gold. Eluted proteins were analyzed using established western blotting techniques, with antibodies to 7 proteins.[2]

Purified apoAI was radioactively labeled with I-125, and its adsorption to biomaterial surfaces over time from plasma diluted to varying extents, was investigated.

Results and Discussion: Proteins bound to Tecothane were identified by immunoblotting. Although albumin, IgG, fibrinogen, and transferring, combined, represent approximately 75% of the protein present in human plasma, only small amounts of these proteins were found on the polyurethane surface following a 2 h exposure to 10% plasma (Figure 1). By comparison, apoAI, present in plasma at a much lower concentration, was a major adsorbate on this, as well as other biomaterial surfaces. This conclusion is supported by the data in Figure 2 showing strong immunoblot responses at plasma concentration as low as 0.1%.

Using the radiolabelled protein as a tracer, apoAI was found to bind rapidly and at high surface concentration (Figure 3) to the Tecothane surface. There is no evidence that this protein is subject to the Vroman effect whereby it would be adsorbed and then replaced by other proteins. This again indicates that apoAI (and/or HDL) is a highly surface active protein.

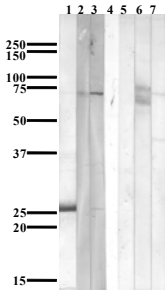

Figure 1: Western blot of proteins eluted from polyurethane following a 2 h exposure to 10% plasma. Lane 1: apoAI, 2: fibrinogen, 3: albumin, 4: IgG, 5: transferrin, 6: vitronectin, 7: C3.

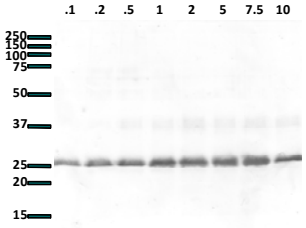

Figure 2: Anti-apoAI immunoblot of protein associated with polyurethane surface after 2 h plasma contact. Plasma at different dilutions from 0.1 to 10%.

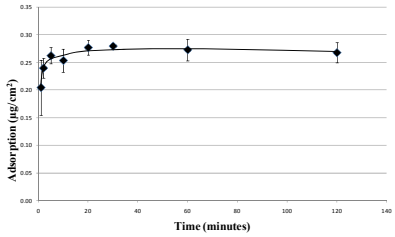

Figure 3: Kinetics of adsorption of apoAI to polyurethane from 10% plasma.

Conclusions: ApoAI has been identified previously as a major protein bound to a wide variety of surfaces. Results presented here indicate that this protein binds rapidly (1 min) and a high surface concentration (~ 0.2 µg/cm^2). If the protein is mainly in HDL-bound form the true surface concentration may be much higher. ApoAI and/or HDL deposition appears to be an important effect in blood-biomaterial interactions. Clearly the binding of this protein warrants further investigation to determine whether its presence on biomaterial surfaces is beneficial or otherwise.

References: [1] Cornelius, RM et al. Biomaterials, 2002: 21: 2583. [2] Cornelius, RM et al. J. Biomed Mater Res, 2003: 67A: 475.

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