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Interfacial energetics of blood plasma and serum adsorption to a hydrophobic self-assembled monolayer surface ☆

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Abstract

Interfacial energetics of blood plasma and serum adsorption to a hydrophobic, methyl-terminated self-assembled monolayer (SAM) surface (solid–liquid SL interface) are shown to be essentially the same as to the buffer–air interface (liquid–vapor LV interface). Specifically, spreading pressure (Π_a) isotherms scaled on a w/v concentration basis constructed from advancing contact angles (θ_a) of serially diluted plasma/serum derived from four different mammalian species (bovine, equine, human, and ovine) on the SAM surface are not resolvable at the 99% confidence level and furthermore are found to be strikingly similar to isotherms of purified human-blood proteins. Maximum advancing spreading pressures Π_a^{max} for protein mixtures fall within a relatively narrow $17 < \Pi_a^{max} < 26$ mN/m band, mirroring results obtained at the LV surface. These observations lead to the conclusion that neither depletion of coagulation proteins in the conversion of plasma to serum nor variation in the plasma proteome among species has a substantial affect on adsorption energetics to these test hydrophobic surfaces. Experimental results are rationalized on the basis that there is a generic mechanism controlling adsorption of globular-blood proteins to test hydrophobic surfaces. We conclude that this generic mechanism is the hydrophobic effect by which proteins are expelled from aqueous solution in order to increase hydrogen-bonding (self-association) among water molecules at the expense of less favorable water–protein interactions. Expelled protein readily displaces water within the hydrophobic-surface region and becomes adsorbed. The amount of water displaced *per gram* of adsorbed protein does not vary greatly among globular proteins because the partial specific volume v^0 of globular proteins is quite conserved ($0.70 \leqslant v^0 \leqslant 0.75\,cm^3/g$ protein). Any single blood protein or mixture of proteins consequently displaces nearly an equivalent amount of interfacial water and hence adsorption is observed to scale similarly with solution concentration expressed in w/v units.

Keywords: Plasma; Serum; Protein adsorption; Hydrophobic solid–liquid interface; SAM

1. Introduction

Perhaps it was Johlin who made the first clear experimental connection between protein adsorption and the biological response to artificial materials by noting in his 1929 classic *Interfacial Adsorption as a Factor in the Clotting of Blood Plasma* [1] that "…clotting can be induced by the contact of the plasma with substances that produce an adsorbing interface…". If not Johlin, it was certainly in the literature of this timeframe that we find origins of a working hypothesis that has emerged over the decades as a fundamental biomaterials-surface-science tenet stating that *protein adsorption is the first step in the biological response to materials*. The focus on protein adsorption has henceforth been unswerving, from the systematic studies of the adsorption behavior of purified proteins by Lyman and Brash [2–6] carried out in the 1965–1975 era, through to today's emphasis on the use of

☆ A Contribution from the Hematology at Biomaterial Interfaces Research Group.

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powerful analytical techniques such as ellipsometry [7], internal-reflection spectroscopy [8], and surface-plasmon resonance [9]; to name a few from many. And for all this effort, the mechanism of protein adsorption to biomaterial surfaces remains an important and controversial topic [10]. Among the important unsolved, outstanding issues are (i) how protein selectively collects at biomaterial surfaces from multi-component protein solutions such as blood; (ii) quantitative structure-property relationships connecting surface chemistry/energy to the extent and specificity of protein adsorption; and (iii) detailed biochemical mechanisms by which surface-bound protein directs the biological response to artificial materials [11].

Our attention has been riveted by recent experimental observations that the adsorption energetics of blood plasma and serum to the hydrophobic, aqueous-buffer/air (liquid–vapor, LV) surface is practically identical among diverse mammalian species (bovine, equine, human, and ovine donors) [12], in spite of substantial differences in plasma proteomes. It has also been found that adsorption of plasma/serum is very similar to that of purified-plasma-protein constituents [13–15]. These results are quite unexpected on the widely held basis that different proteins should adsorb differently to the same adsorbent surface. Indeed, if different proteins adsorb to the same surface in significantly differently ways (e.g. more-or-less surface concentration, more-or-less strongly, or more-or-less reversibly), then interfacial energetics of adsorption should reflect these differences because interfacial energetics are exquisitely sensitive to these aspects of adsorption [15–20]. Instead, we find "generic" interfacial energetics of protein adsorption to the LV surface and interpret this evidence in terms of a heretofore unresolved mechanistic commonality underlying protein adsorption [12]. Interesting as this hint of an underlying commonality might be, the LV surface is, after all, not entirely germane to biomaterials and might be unique as an adsorbent surface.

With all of the above in mind, we have extended our protein-adsorption studies to methyl-terminated, self-assembled monolayers (SAMs) supported on gold-coated semi-conductor wafers (solid–liquid, SL interface) as a model hydrophobic solid surface. These studies show that adsorption energetics of purified-plasma proteins spanning 3 decades in molecular weight (MW) is very similar to that observed at the LV surface [11,16], suggesting that the mechanism of protein adsorption to this hydrophobic SL surface is not fundamentally different than adsorption to the LV surface. Herein we report that adsorption behavior of these purified proteins cannot be clearly distinguished from the adsorption of plasma and serum, again mirroring results obtained at the LV surface reported above. Experimental observations are reconciled by asserting that the hydrophobic effect is the mechanistic commonality underlying protein adsorption to hydrophobic surfaces.

2. Methods and materials

2.1. Plasma/serum

Human platelet-poor plasma (citrated) was prepared from outdated (within 2 days of expiration) lots obtained from the Hershey Medical Center Blood Bank. Human serum was prepared from this plasma in 15 mL batches by recalcification with 0.1 M $CaCl_2$ at 5:1 v/v plasma:calcium ratio in clean glass scintillation vials for about 15 min. Bovine, ovine, and equine plasma and serum were used as received from Hemaresource and Supply Inc. (Aurora, OR) and were not subjected to any additional fractionation/purification steps. Ref. [13] discloses details of protein solution preparation including serial dilutions that were performed in 96-well microtiter plates by (typically) 50:50 dilution in phosphate buffered saline solution (PBS) prepared from powder (Sigma Aldrich) in distilled-deionized (18 MΩ) water (interfacial tension of PBS and water was checked periodically by Wilhelmy-balance tensiometry).

2.2. Surfaces

Methyl-terminated, thiol-based self-assembled monolayer surfaces (SAMs) on gold-coated electronic-grade semiconductors were prepared according to standard methods of surface engineering as reported elsewhere [11,16,21–26]. Briefly, silicon wafers were pre-cleaned in hot 1:4 H_2O_2 (30%)/H_2SO_4 followed by rinsing with distilled-deionized H_2O and absolute ethanol. Gold-coated wafers were prepared by vapor deposition of chromium and gold (99.99% purity) from resistively heated tungsten boats onto clean 3-in. diameter silicon wafers at about 1×10^{-8} Torr base pressure in a cryogenically pumped deposition chamber. The sample was not allowed to rise above ~40 °C during the evaporation. Film thicknesses, monitored with a quartz crystal oscillator, were typically 15 and 200 nm for chromium and gold, respectively. Chromium was deposited prior to gold to enhance adhesion to the substrate. After deposition, the chamber was backfilled with research-grade nitrogen. Gold-coated samples were removed and immersed in 1 mM solutions of 1-hexadecanethiol ($CH_3(CH_2)_{15}SH$) in ethanol, contained in glass jars at ambient temperature, for at least 3 days. The alkanethiol (Aldrich Chemical Co., Milwaukee, WI) and ethanol (commercial reagent-grade) were used as-received, without further purification. Samples were stored in the thiol solution until use, rinsed with ethanol and air dried just prior to an experiment.

2.3. Tensiometry and goniometry

Liquid–vapor interfacial tensions required by this work were measured by pendant drop tensiometry (PDT) as described in Refs. [12–15]. Contact angle and wettability methods applied in this work have been disclosed in detail elsewhere, including verification that measured advancing angles (θ_a) were in statistical agreement with that obtained by Wilhelmy balance tensiometry [16,21]. Receding angles (θ_r) were shown to be not as reliable as θ_a. Consequently, only θ_a was analyzed in this work. Briefly, for the purposes of this paper, θ_a measurements were made using a commercial automated tilting-plate goniometer (TPG, First Ten Angstroms Inc., Portsmouth VA). The goniometer employed a Tecan liquid-handling robot to aspirate 12 μL of solutions contained in a 96-well microtiter plate prepared by the serial-dilution protocol mentioned above. The robot was used to reproducibly transfer the tip with fluid contents into a humidified (99 + % RH) analysis chamber and dispense10 μL drops of protein solution onto the surface of test substrata held within the focal plane of a magnifying camera. These and all other aspects of tilting-plate goniometry were performed under computer control. Proprietary algorithms supplied by the vendor were used to deduce contact angles from drop images captured at a programmed rate by a frame grabber. Typically, 600 images were captured at a rate of 1 image every 6 s following 20 s delay to permit vibrations of the expelled drop to dampen. Precision of θ_a was about 0.5° based on repeated measurement of the same drop. The analysis chamber