INTERACTION OF HUMAN FIBRINOGEN WITH PURE LTI-CARBON*

T-H. Chiu and E. Nyilas
Avco Everett Research Laboratory, Inc.
Everett, MA 02149

Low temperature isotropic (LTI) carbon surfaces which have been diamond-polished according to specified tolerances, display a favorable degree of non-thrombogenicity not only in vitro and ex vivo but, most importantly, in vivo as parts of various prosthetic devices chronically implanted in man. As the primary event of their interaction with native blood, foreign surfaces acquire within very short periods of time a "conditioning" layer that is a few hundred Å's thick and composed of sorbed plasma proteins. This layer mitigates the effects of the original contact surface on other blood components and hence, the state of protein molecules in it (viz., native, partially or irreversibly denatured) can influence the incidence of potentially ensuing thromboembolic phenomena. Based on experimental evidence [1] consistent with diffusion kinetic theory, [2] fibrinogen (FGN) which has been strongly implicated in thromboembolic phenomena, can be expected to be a major constituent of the "conditioning" protein layer. With respect of these factors, the studies reported here have been undertaken to elucidate the specifics of the mechanism and energetics of interaction between native FGN and LTI-carbon surfaces.

Microparticulate LTI-carbon adsorbent was obtained from discs, prepared at the Medical Products Div., General Atomic Co., by the deposition of this type of carbon on ultrathin graphite substrates. After the grinding of these discs in nonmetallic equipment over extended periods of time, the powder collected consisted of polydisperse particles which $\leq 1\mu$, contained 97.3% pure LTI-carbon, and gave a specific surface area of 109.2 m^2/g as determined by B.E.T. multipoint N_2 adsorption at -195°C. This area was inconsistent with the particle size observed in SEM's, and indicated the presence of porosity which was consistent with the established internal micromorphology of LTI-carbon.[3] Using (a) the measured hysteresis between the 25°C water vapor adsorption and desorption isotherms of the LTI-carbon adsorbent, and (b) the pressurized mercury penetration technique,[4] the pore size and shape distribution was determined revealing the presence of "ink-bottle" type pores. Analysis of these data gave 27.7 m^2/g as the specific surface area of the LTI-carbon adsorbent, which is exclusive of the internal surface area constituted by "ink-bottle" pores and is unrestrictedly accessible to all types of molecules, regardless of their size and shape. This area is commensurate with the particle size observed, and has been taken as the effective specific surface area for protein sorption. As described in a companion paper[5] the LTI-carbon adsorbent was also characterized by its (a) 25° and 37°C heats of immersion into a standard buffer, $h_I(SLB)_T$, whose composition is specified below, and (b) electrophoretic mobilities determined in the same buffer at the same temperature.

All FGN used in these experiments was freshly purified according to the method of Laki,[6] had a clottability of $\geq 92\%$; and displayed upon sodium dodecyl sulfate (SDS)/polyacrylamide gel electrophoresis only 3 bands, as required, corresponding to the α-, β- and γ-chains of the molecule which were detectable by staining with Coomassie-blue. In all experiments reported here, FGN was (a) adsorbed from a standardized sodium acetate/HCl buffer having a pH= 7.4 which simulated plasma conditions, and a low ionic strength of 0.05; and (b) at a fixed ratio of total available adsorbent surface area to total protein solution volume, $\Sigma'/V = 277\ cm^2/ml$.

The 25° and 37°C adsorption isotherms of FGN on the microparticulate LTI-carbon adsorbent were determined according to experimental protocols described elsewhere.[2, 7] Both isotherms have indicated multilayer sorption with FGN adsorptivities at the completion of first monolayer coverage, which were found to be identical within the error of the measurement at both temperatures. Based on the specific protein sorption area given above, the first monolayer coverages obtained convert to $\sigma_1(25°) \simeq \sigma_1(37°) \simeq 0.52 \mu g/cm^2$.

In another series of experiments also employing the experimental conditions specified earlier, the 25° and 37°C heats of FGN sorption on the LTI-carbon powder adsorbent were directly measured as a function of increasing bulk protein concentrations. This was performed with a custom-made, isothermal-jacketed, thermistorized microcalorimeter system routinely capable of resolving $\pm 1 \times 10^{-5}$°C in 100 ml of liquid volume.[2, 7] As a function of increasing surface coverages, the 25°C integral net heats released per mole of sorbed FGN have remained approximately constant whereas those obtained at 37°C have shown a moderate decrease within the concentration range studied. At the completion of first monolayer coverages, the integral net heats of FGN sorption on the microparticulate LTI-carbon are -3.6×10^3 and -2.4×10^3 Kcal/mole at 25° and 37°C, respectively. These quantities are smaller, by a factor of about 5, than the integral net heats of sorption of FGN reported elsewhere,[8] which have been determined under identical conditions but on a microparticulate glass adsorbent. In addition, the inverse holds for the FGN/glass system inasmuch as its 37°C integral net heats of sorption are greater than those obtained at 25°C. Thus, the different trends in the temperature-dependence of FGN sorption as well as the large difference between the magnitudes of the heats of sorption of that protein at comparable surface coverages indicate an entirely different structuring and interaction mechanism of FGN sorbed onto LTI-carbon surfaces.

The 25° and 37°C electrophoretic mobilities of LTI-carbon adsorbent particles were measured, which were coated with known amounts of FGN adsorbed at the corresponding temperatures under the conditions specified earlier. At both temperatures, these mobilities have remained practically

*This work was performed with support from the Biomaterials Program, Devices and Technology Branch, Division of Heart and Vascular Diseases, National Heart, Lung, and Blood Institute, under Contract NIH-N01-HV-3-2917.

constant, at about $-1.5\mu\text{-sec}^{-1}\text{-V}^{-1}$ cm, up to a relative surface coverage, $\theta \sim 0.7$, and levelled off to a steady-state value at about first monolayer completion. This trend of change in the mobilities of FGN-coated LTI-carbon particles is in sharp contrast to that displayed by particles of the strongly procoagulant glass,[8] which were also coated with the same protein under identical conditions but rapidly shifted their mobilities in a positive direction with increasing θ's. The rapid positive shift indicates that sorbed FGN molecules effectively cancel negative charges on the glass surface. This, in turn, implies that FGN molecules undergo restructuring on glass, in contrast to that observed on LTI-carbon on which the original charge is preserved suggesting, at least, a less severe restructuring of the sorbed protein. Thus, the electrophoretic mobility data of FGN-coated LTI-carbon particles are in concert with the trend shown by the integral net heats of sorption of that protein on the same adsorbent.

The intensity of interaction arising between a native protein and an adsorbent, as measured in terms of the integral net heat of protein sorption, is generally a function of the mean number of binding sites established per sorbed protein molecule, and the mean net binding energy per site. Relatively smaller integral net heats of sorption of a given protein at submonolayer coverages indicate, as a minimum, either a relatively smaller number of attachment sites or smaller mean binding energies per site, or both. According to the working hypothesis underlying these studies, which was put forward earlier,[2] native plasma proteins may retain their conformation or, undergo relatively less extensive adsorption-induced structural rearrangements on nonthrombogenic surfaces than on thrombogenic ones. This implies that, under identical experimental conditions, the calorimetrically determinable interaction energy between a given native protein and different surfaces should be smaller for the less thrombogenic materials. In comparison to the FGN/glass system,[8] both the microcalorimetric and electrophoretic mobility data described above consistently indicate that native FGN undergoes, at least at submonolayer coverages, a considerably less intense interaction with the relatively nonthrombogenic surface of LTI-carbon. The data obtained for the microparticulate LTI-carbon and glass adsorption systems also indicate that, at least these 2 systems, support the postulations of our working hypothesis and imply a significantly smaller degree of adsorption-induced restructuring of native FGN on LTI-carbon surfaces. The same data which pertain to interactions occurring at the molecular level, appear to be consistent with the nonthrombogenic properties of LTI-carbon surfaces displayed on a macroscopic scale.

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