ADSORPTIVE PROPERTIES OF HUMAN γ-GLOBULIN ON PURE AND SILICON ALLOYED LTI-CARBONS*

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As part of a study employing model systems to elucidate the energetics and mechanisms of native plasma protein sorption, the adsorptive properties of human γ-(7S)-globulin (γ-GLB) were investigated earlier with glass, one of the strongest known procoagulants.[1, 2] This work has been extended to low temperature isotropic (LTI) and silicon-alloyed (SLTI) carbons, both of which are relevant biomaterials.

Microparticulate adsorbents composed of either 97.3% pure LTI-carbon or, 96.7% pure SLTI-carbon containing on the order of 10% by wt. silicon, were obtained from discs which were prepared at the Medical Products Div., General Atomic Co. by deposition of the corresponding types of carbon on ultrathin graphitic substrates. After grinding over extended periods of time in nonmetallic equipment to avoid any contamination, both types of discs yielded polydisperse particles measuring $\leq 1\mu$ in the SEM. For both adsorbents, B.E.T. multipoint N_2 adsorption at -195°C gave specific surface areas (Σ_{BET}) listed in Table I. These values are disproportionately greater than the specific surface areas which can be estimated from the particle sizes observed, indicating the presence of internal porosity. For LTI-carbon adsorbent, this is consistent with its established internal micromorphology.[3] Based on the (a) hysteresis between its 25°C water vapor adsorption and desorption isotherm, and (b) mercury penetration volume technique,[4] the pore size and shape distribution of each adsorbent has been determined. Analyses of these data revealed that both powders contain "ink-bottle" pores, and yielded the values, denoted as Σ_{eff} in Table I, as the specific surface areas exclusive of the internal surface areas of "ink-bottle" pores. These have been taken as the areas effective in protein sorption. Both the LTI- and SLTI-carbon adsorbents have been characterized, at T = 25° or 37°C, by their (a) heats of immersion into pure water, $h_I(SLW)_T$; (b) heats of immersion into a standardized sodium acetate/HCl buffer whose composition is given below, $h_I(SLB)_T$; and (c) electrophoretic mobilities, $u_e(25^o)$ and $u_e(37^o)$, with the 37°C data corrected to 25°C, $u_e'(37^o)$. For both adsorbents, these data are given in Table I.

TABLE I

$\Sigma_{BET}(m^2/g)$	109.2	52.8
$\Sigma_{eff}(m^2/g)$	27.7	17.8
$\Sigma_{BET}/\Sigma_{eff}$	3.9	3.0
$h_I(SLW)_{25}$ (erg/cm^2)*	-66.7±12.3	-51.5±3.5
$h_I(SLW)_{35}$ (erg/cm^2)*	-62.6±4.7	-52.6±3.5
$h_I(SLB)_{25}$ (erg/cm^2)*	-83.5±11.1	-60.4±2.3
$h_I(SLB)_{37}$ (erg/cm^2)*	-87.7±3.5	-65.8±1.8
$u_e(25^o)$ (μ-sec^{-1}-V^{-1}cm)	-1.63±.17	-1.46±.20
$u_e'(37^o)$ (μ-sec^{-1}-V^{-1}cm)	-1.45±.24	-1.58±.15

*Values are based on the corresponding Σ_{BET}.

All γ-GLB used was the highest commercially available grade 95% pure by electrophoresis. In all experiments, γ-GLB was always exposed to the adsorbents in a standardized sodium acetate/HCl buffer having a pH = 7.4 which simulated plasma conditions, and a low ionic strength of 0.05. In each protein adsorption, a fixed ratio of total available surface area to total protein solution volume, Σ'/V was used for each of the adsorbents. This was 277 cm^2/ml for the LTI-carbon, and 178 cm^2/ml for SLTI-carbon. The ratio given for LTI-carbon is identical to that used in human fibrinogen adsorption studies on that material, which have been reported in a companion paper.[5]

Determination of the 25° and 37°C γ-GLB adsorption isotherms of the microparticulate LTI- and SLTI-carbons was performed according to established protocols described elsewhere.[1, 2] All enthalpy changes, including $h_I(SLW)_T$'s, $h_I(SLB)_T$'s and the 25° and 37°C heats of immersion of each of the adsorbents into γ-GLB solutions containing known amounts of the protein, i.e., $h_I(SLP)_T$'s were directly measured using a custom-made, isothermal-jacketed, thermistorized microcalorimeter system which, as reported elsewhere,[1, 2] is routinely capable of resolving $\pm$ 1 x 10^{-5}°C in 100 ml of liquid volume. All electrophoretic mobility determinations were performed in the standard buffer, using a calibrated precision-bore glass capillary cell with a ground optical published flat. In each of these measurements, the mobilities of an average of 12-14 particles were recorded in both directions. All mobility values obtained at 37°C have been corrected to 25°C.

In the concentration range (0 - 2.0 mg/ml) studied, both the 25° and 37°C adsorption isotherms of γ-GLB on the microparticulate LTI-carbon indicate multilayer sorption. This is confirmed by the fact that a Langmuir plot of each of these isotherms splits into 2 linear segments having different slopes and intercepts. Both isotherms display well-defined "knee" points which are indistinguishable within the error of the measurement, and correspond to the completion of first monolayer coverages. Using the Σ_{eff} of LTI-carbon, these coverages convert to $\bar{\sigma}_1(25^o) \simeq \bar{\sigma}_1(37^o) \simeq 0.23\mu g/cm^2$. On the microparticulate SLTI-carbon, the shape of the 25°C isotherm of γ-GLB clearly displays multilayer sorption easily confirmed by the corresponding Langmuir plot. In contrast, the 37°C isotherm does not exhibit a well-defined "knee" point but the corresponding Langmuir plot verifies the incidence of multilayer sorption at that temperature as well. Based on the Σ_{eff} of SLTI-carbon, adsorbances at the completion of first γ-GLB monolayers are $\bar{\sigma}_1(25^o) \simeq 0.28\mu g/cm^2$ and $\bar{\sigma}_1(37^o) \simeq 0.24\mu g/cm^2$, which are indistinguishable from each other as well

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as from the $\bar{\sigma}_1$'s obtained for the pure LTI-carbon. Thus, no appreciable differences in γ-GLB adsorptivity appear between the LTI- and SLTI-carbons.

A measure of the intensity of interaction between a native protein and a given adsorbent is the integral net heat of protein sorption which, at a T temperature, is $\Delta\bar{H}' = h_I(SLP)_T - h_I(SLB)_T$. In general, $\Delta\bar{H}'$ is a function of the mean number of binding sites established per sorbed protein molecule, and the mean net binding energy per site. Since the term $h_I(SLP)_T$ measures the enthalpy change of protein attachment in the presence of buffer ions and since $h_I(SLB)_T$ measures that of the buffer alone, both processes are exothermic and hence, the sign of both quantities is negative. If $|h_I(SLP)_T| > |h_I(SLB)_T|$, $\Delta\bar{H}'$ is also negative.

For both microparticulate LTI- and SLTI-carbons, the calorimetrically measured values of $|h_I(SLP)_T| < |h_I(SLB)_T|$ when these adsorbents are exposed, at either 25° or 37°C, to γ-GLB solutions which have bulk protein concentrations (C_o) up to 0.10 mg/ml and give rise to coverages ranging up to ~ 0.20 μg/cm^2 (or relative γ-GLB surface coverages up to $\theta \sim 0.75$). Under these conditions, positive $\Delta\bar{H}'$ values are obtained which would seemingly indicate that the protein attachment in itself would be endothermic. However, direct attachment of the protein involves the displacement of water and ions from the surface, and the enthalpy change associated with protein attachment can, in certain cases, be smaller than the enthalpy change that is associated with the water and ion displacement. In these cases, attachment of protein molecules is attained if the entropy change of the overall adsorption process is positive. It can be shown that, in cases where nominally positive of $\Delta\bar{H}'$ have been obtained at submonolayer coverages on both LTI- and SLTI-carbons, the integral net heats of γ-GLB sorption are negative (i.e., exothermic), and can be computed using $\Delta\bar{H}' = h_I(SLP)_T - h_I(SLB)_T\ (1-\theta\Sigma_{eff}/\Sigma_{BET})$.

At C_o's > 0.10 mg/ml, $|h_I(SLP)_T| > |h_I(SLB)_T|$ and hence, all $\Delta\bar{H}'$ values are negative for both adsorbents. Within the entire concentration range studied, the magnitude of all integral net heats of sorption are in the range of -(2-4) x 10^2 Kcal/per mole of sorbed γ-GLB. At θ=1, the 37°C integral net heats of γ-GLB sorption on the LTI- and SLTI-carbons are, respectively, -356 and -328 Kcal/mole. At comparable surface coverages, these heats are somewhat smaller for the SLTI-carbon, consistent with the fact that, as described in a companion paper,[6] the SLTI surface is relatively "hydrophobic" as compared to that of the LTI-carbon. This is also reflected by their respective $h_I(SLW)_T$ values listed in Table I.

The adsorptive properties displayed by γ-GLB on LTI- and SLTI-carbons are in sharp contrast to those displayed by the same protein under identical conditions but on a microparticulate glass. As described elsewhere,[1,2] the 25° and 37° integral net heats of γ-GLB on the glass adsorbent are respectively, -1,700 and -1,200 Kcal/mole at completion of first monolayer coverages, which are an order of magnitude greater than those obtained on the LTI- and SLTI-carbons. This indicates, at least, that γ-GLB undergoes an entirely different interaction mechanism with glass.

The electrophoretic mobilities of LTI- and SLTI-carbon particles were measured at 25° and 37°C after they were coated with known amounts of γ-GLB adsorbed under the conditions specified earlier. The mobilities of both types of protein-coated carbon particles remained equal to those of the corresponding uncoated particles up to $\theta \sim 0.85$, and levelled off to a steady-state value at θ=1. In contrast, particles of the strongly procoagulant glass, which were also coated with γ-GLB under identical conditions, rapidly changed their mobilities in a positive direction at relatively small surface coverages ($\theta \leq 0.1$), indicating that small amounts of the sorbed protein are already sufficient to neutralize negative charges on the glass surface.

The trend of change in the mobilities of γ-GLB-coated LTI- and SLTI-carbon particles as well as the comparatively small values of the integral net heats of γ-GLB sorption on these carbons are consistent with each other. In addition, both sets of data indicate that, at least at submonolayer coverages, γ-GLB undergoes a significantly smaller degree of adsorption-induced restructuring on both carbon surfaces. With respect to the definition of the integral net heat of protein sorption, relatively smaller values of this enthalpy quantity are indicative of less intense interactions which occur between a particular native protein and various adsorbents. As described earlier,[1,2] the working hypothesis of the studies reported here holds that, in contrast to procoagulants, nonthrombogenic surfaces give rise to less severe adsorption-induced structural alterations in sorbed plasma proteins. As characterized in terms of the microcalorimetric and electrophoretic mobility data given above, the adsorptive properties of γ-GLB on LTI- and SLTI surfaces are in concert with the established nonthrombogenic properties of these carbons, and support our working hypothesis.

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