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Published on Web 03/07/2006

Surface Tailoring for Controlled Protein Adsorption: Effect of Topography at the Nanometer Scale and Chemistry

Paul Roach,[†] David Farrar,[‡] and Carole C. Perry*,[†]

Contribution from the Division of Chemistry, Interdisciplinary Biomedical Research Centre, School of Biomedical and Natural Sciences, Nottingham Trent University, Clifton, Nottingham, U.K., and Smith and Nephew Group Research Centre, Heslington, York, U.K.

Received September 19, 2005; E-mail: Carole.Perry@ntu.ac.uk

Abstract: Protein adsorption behavior is at the heart of many of today's research fields including biotechnology and materials science. With understanding of protein−surface interactions, control over the conformation and orientation of immobilized species may ultimately allow tailor-made surfaces to be generated. In this contribution protein−surface interactions have been examined with particular focus on surface curvature with and without surface chemistry effects. Silica spheres with diameters in the range 15−165 nm with both hydrophilic and hydrophobic surface chemistries have been used as model substrates. Two proteins differing in size and shape, bovine serum albumin (BSA) and bovine fibrinogen (Fg), have been used in model studies of protein binding with detailed secondary structure analysis being performed using infrared spectroscopy (IR) on surface-bound proteins. Although trends in binding affinity and saturation values were similar for both proteins, albumin is increasingly less ordered on larger substrates, while fibrinogen, in contrast, loses secondary structure to a greater extent when adsorbing onto particles with high surface curvature. These effects are compounded by surface chemistry, with both proteins becoming more denatured on hydrophobic surfaces. Both surface chemistry and topography play key roles in determining the structure of the bound proteins. A model of the binding characteristics of these two proteins onto surfaces having differing curvature and chemistry is presented. We propose that properties of an adsorbed protein layer may be guided through careful consideration of surface structure, allowing the fabrication of materials/surface coatings with tailored bioactivity.

Introduction

Protein adsorption is of relevance to a wide audience due to its many possible applications, especially with the increasing interest in bio-nanotechnology. The interaction of proteins on surfaces is of crucial importance in biochip developments,[1−3] biosensors,[4] medical device coatings,[5] drug delivery,[6] and even the fabrication of a new class of hybrid materials.[7] Immobilization of proteins such as antibodies or enzymes, and conservation of their activity, may give rise to functional surfaces suitable for use in many fields including biosensors and the development of biocompatible materials. However, the protein−surface interactions that govern the behavior of immobilized protein molecules are not well-established.

Protein adsorption characteristics can be controlled by changing substrate surface parameters such as chemistry,[7−12] size, and curvature.[13,14] However, the extent to which each of these factors contributes to the control of conformation and thus the activity of surface-bound proteins is not fully understood, although clearly being of major importance to biocompatibility.[12] Furthermore, studies examining the effect of surface topography (through surface curvature) have focused on small globular proteins such as lysosyme (MW = 14.5 kDa, 4.5 × 3.5 × 3.5 nm)[13,15] and cytochrome C (MW = 15 kDa, 2.5 × 2.5 × 3.7 nm).[9] Here we broaden the knowledge base by investigating the effects of surface chemistry with and without surface curvature, using two model proteins: bovine serum albumin (BSA), a small globular protein, and bovine fibrinogen (Fg), a rodlike protein. Both are of significant relevance not only as model systems but also in biocompatibility and drug delivery

[†] Nottingham Trent University.
[‡] Smith and Nephew Group Research Centre.

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applications due to the high abundance of these proteins at implant sites.

A large number of investigations examining bound proteins have used techniques that give only limited information. Circular dichroism (CD) for instance is one of the more common methods used to study protein adsorption onto colloidal particles; although changes in secondary structure can be followed, this technique has inherent inconsistency problems in absolute secondary structure determination.[16] Colloidal scattering also restricts the size of substrates that can be used, which obviously limits the range of investigations using this technique.[13] Infrared spectroscopy (IR) has been used in this study, allowing a more thorough examination of the bound protein secondary structure[8,17−18] as it is a method not constrained by substrate size or material. Previous studies examining how surface chemistry affects protein adsorption have focused on the effects of surface chemistry alone, most taking little account of surface roughness on the nanoscale that can be experienced by adsorbing proteins. This is the first study examining how proteins of differing size and shape (albumin and fibrinogen) interact with surfaces having defined chemistry and topography.

Substrates having diameters in the range 15−165 nm have been synthesized and chemically modified to give model hydrophilic (hydroxy-terminated) and hydrophobic (alkane-terminated) surfaces. It was the intention that the particles would coincide with protein dimensions. In relation to the sizes of the protein molecules used, the smaller substrates present high surface curvature while the larger substrates are observed as pseudo-flat surfaces. Infrared spectroscopy has been used to assess, in detail, the conformation and also orientational changes of the bound proteins. Due to the high substrate surface area used, it was also possible to measure protein loss from solution directly via ultraviolet (UV) spectroscopy. Together, these complimentary techniques have been used to elucidate the adsorption characteristics of two serum proteins.

Materials and Methods

Bovine fibrinogen (Fg, type I-S, lyophilized powder) and bovine serum albumin (BSA, fraction V, lyophilized powder) were obtained from Sigma and used as received. Phosphate buffered saline (PBS) was freshly prepared using sodium salts: NaH_2PO_4 and Na_2HPO_4 (200 mmol of phosphate) and NaCl (100 mmol) obtained from Aldrich to give pH 7.4 at 25 °C. Protein solutions with concentrations in the range 0.01−2.70 g L^{-1} were prepared from 2 to 4 g L^{-1} stock solutions immediately before use. Distilled, deionized water with a conductivity of ∼1 μS was used for all experiments.

Preparation of Silica Spheres. Monodisperse silica spheres of varying sizes were prepared via the Stöber process.[19] Briefly, batches of spheres were prepared by the addition of ammonia to ethanolic solutions of tetraethoxyorthosilicate. These were stirred overnight and left to stand at 25 °C for more than 5 days. Spheres were collected by centrifugation and acid-treated using 1 N HCl (Fisher) to ensure all terminal ethoxy groups were converted to hydroxyl groups. Silica spheres were washed by repeated sonication and centrifugation cycles until the water removed had a conductivity of ∼5 μS. Transmission electron microscopy (TEM) was used to measure particle sizes. A minimum of 40 particles for each sample were measured. Surface area measurements were performed by nitrogen adsorption analysis using a Quantachrome Nova3200 system after degassing the samples overnight under vacuum at 120 °C. Methyl-terminated spheres were produced by silylating hydroxyl-terminated particles with a vast excess of chlorotrimethylsilane (31.5 mmol, 98% Aldrich) in toluene (Fisher), stirring overnight, and washing in toluene. The surface chemistry of the silica particles as prepared was analyzed by infrared spectroscopy using a Nicolet Magna IR-750 spectrometer fitted with a Golden Gate attenuated total reflection (ATR) accessory (Thermo Nicolet). Spectra were recorded at 4 cm^{-1} resolution, averaging 256 scans. Surface wettability was characterized by measuring equilibrium water contact angles on a Krüss DSA10. A 5 μL water droplet was deposited onto lose powdered samples by microsyringe and images were taken immediately to eliminate drying effects.

Evaluation of Protein Adsorption Isotherms. Batch experiments were conducted at 25 °C and ambient pressure. A range of concentrations (0.1−3.0 mg mL^{-1}) of BSA and Fg in PBS were prepared. Silica spheres were sonicated in PBS to form homogeneous dispersions prior to the addition of protein. With stirring the silica suspension was added to each protein solution to give a total substrate surface area per solution of ∼0.2 m^2 mL^{-1}. Solutions were stirred for 1 h and left to stand for a further 2 h to minimize any sheering effects due to stirring. After this time, solutions were centrifuged to induce sedimentation of the silica. To rule out the possibility of protein sedimentation, a blank experiment with protein only was carried out. Residual protein concentration in solution was determined using a Unicam UV2 UV−Vis spectrometer using a quartz cell with 1 cm path length, measuring absorbance at 280 nm. Calibration was carried out using a series of protein solutions of known concentration. Each experiment was performed in triplicate. The silica particles were collected and rinsed with PBS to remove any excess protein solution.

Conformational Assessment of Surface-Bound Proteins. Infrared analysis of surface-bound protein was conducted using a Golden Gate ATR accessory in a Nicolet Magna IR-750 spectrometer continuously purged with dry air. Spectra were recorded at 4 cm^{-1} resolution with 512 scans being averaged, smoothed by 9 point adjacent averaging and curve-fitted. The fitting procedure was such that the amide I band was treated, having a linear baseline between 1720 and 1590 cm^{-1} fitting component peaks with a Gaussian band profile. Peak positions were estimated from the literature,[18,20−25] although not fixed during fitting.

Results and Discussion

Silica particles were formed via a modified Stöber process[19] to afford batches of monodisperse spheres having diameters in the range 15−165 nm, chosen to coincide with protein dimensions. Analysis by IR showed that, after initial workup, terminal ethoxy groups were still present on the silica spheres so an acid treatment step was incorporated in the synthesis, Figure 1.

Nitrogen adsorption analysis allowed determination of the surface area for each batch of spheres; results are listed in Table 1.

Further treatment with chlorotrimethylsilane allowed both hydrophilic and hydrophobic spheres of the same size to be available for further experimentation. Surface chemistry was checked by IR, Figure 1. The ability of a water droplet to spread over a surface was used to assess the wettability of the spheres.[26] Contact angles shown as inserts in Figure 1 demonstrate the

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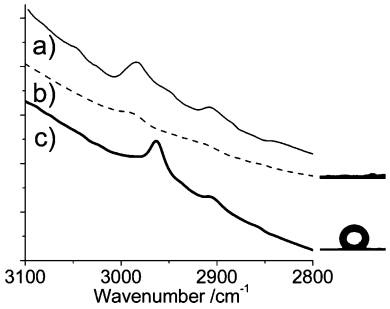

Figure 1. Infrared spectra of C−H stretching region of silica particles: (a) pre-acid, (b) post-acid treatment, and (c) after further methyl termination. Inserts adjacent to corresponding spectra show water droplets placed on hydrophilic and hydrophobic spheres.

Table 1. Spheres Prepared for Protein Adsorption Experiments

sphere radius[a] (nm)	surface area ($m^2\ g^{-1}$)
7.5 ± 0.2	373 ± 2.0
9.6 ± 0.4	350 ± 1.8
14.4 ± 2.1	327 ± 1.7
30.6 ± 1.6	174 ± 0.9
41.0 ± 1.8	81 ± 0.4
81.8 ± 3.9	65 ± 0.3

[a] From TEM measurements.

difference in wettability observed for the treated substrates. The water droplet rested on the silylated spheres, showing that they were hydrophobic, in contrast to behavior on the hydrophilic samples where the water droplet rapidly spread over the surface and entered the space between the spheres.

Protein Surface Studies. To examine the effects of topography on protein adsorption, a range of nanosized spheres were used as substrates, employing surface curvature as a model of topography. It was the intention that protein molecules adsorbing onto the largest spheres would experience a pseudo-flat surface, whereas those adsorbing onto the smaller spheres, being comparable in size to protein dimensions, would experience a curved surface. Proteins with significant differences in their size and shape were chosen as models for investigation.

Albumin, a multifunctional transporter molecule, is the most abundant protein in the circulatory system, having a molecular weight of 66 kDa and dimensions $\sim 14 \times 4$ nm. Several investigations examining the secondary structure of albumin give somewhat contradictory data with some researchers, indicating predominantly α-helical (50−68%) structure with the remaining being made up of β-sheet (16−18%).[27−29] Others suggest the protein has a larger fraction of extended flexible regions and no β-sheet structure.[30]

Fibrinogen is another important plasma protein being involved in the blood clotting process. This protein is much larger than albumin, 340 kDa in mass and 46×4 nm[31] in dimension. Data suggest its secondary structure is comprised of helical (35%), β-sheet (21%), and β-turn (13%) with the remaining being random chains.[32]

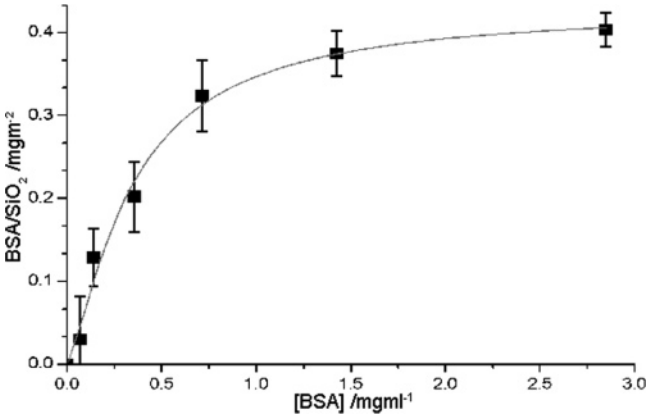

Figure 2. Example saturation curve for BSA adsorbing onto hydrophobic substrates with radii of 82 nm. Error bars show standard deviation.

Affinity and Saturation Levels. All experiments conducted were normalized to surface area to eliminate problems associated with available free protein adsorption sites. Due to the high surface area of the substrate, the difference in free protein concentration before and after incubation was easily observed by a reduction in absorbance at 280 nm. The range of concentrations used was well within the detection limits of the instrument.

The shape of an adsorption isotherm can yield information about the affinity of an adsorbing species toward the surface as well as the maximum equilibrium surface concentration of adsorbed protein. As found in a previous study, surface protein concentrations reach equilibrium after approximately 1 h of incubation.[8]

With fitting of the data to a Langmuir curve, estimates of affinity and the maximum amount of protein adsorbed can be found from the initial slope and plateau, respectively. An example of the data obtained for the saturation curve of BSA onto hydrophobic substrates with radii of 82 nm is shown in Figure 2. The binding constant is related to the equilibrium between adsorbing and desorbing species and directly correlates with the affinity of the adsorbate to the adsorbent, a higher value indicating a stronger attraction.

Figure 3 details the affinity constants and saturation values for BSA and Fg adsorbing onto hydrophilic and hydrophobic substrates of varying sizes.

In general, with decreasing substrate size, i.e., higher surface curvature, the binding affinity of BSA increased with the saturation values following an opposite trend. However, the binding affinities for both albumin and fibrinogen adsorbing onto the largest sphere size do not follow this trend and we cannot explain this inconsistency. In a fashion similar to that found in a previous investigation comparing "flat" surfaces of different wettabilities,[8] albumin typically adsorbed in lower quantities onto hydrophobic compared to hydrophilic surfaces tested. Figure 4 compares protein saturation values obtained experimentally with those predicted for a close-packed monolayer of each protein. Experiments were conducted keeping total substrate surface area constant, and as all size and concentration parameters are known (of both substrate and protein molecules), the number of protein molecules adsorbing per substrate sphere can be calculated. Albumin adsorbs in lower amounts to all

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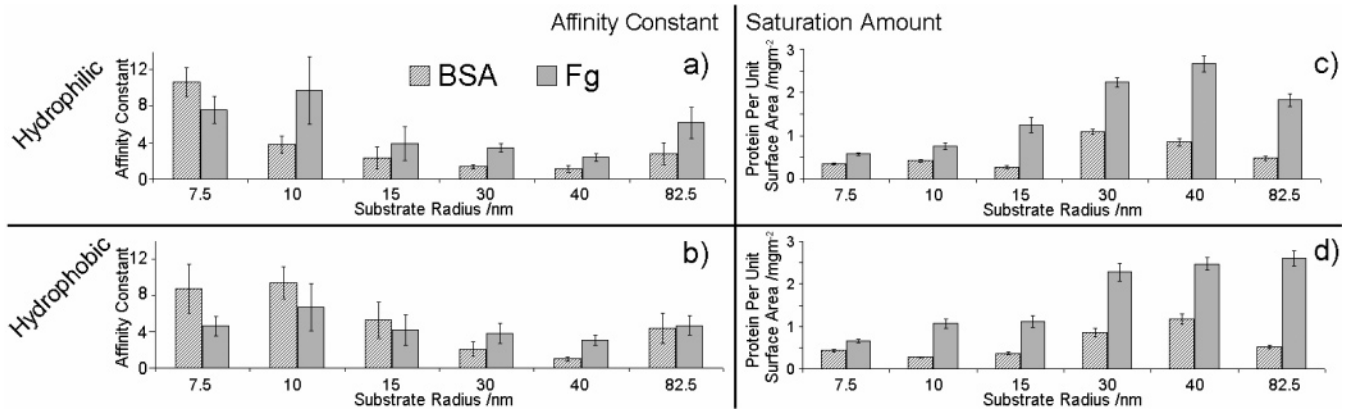

Figure 3. Plots showing (a) and (b) affinity constants and (c) and (d) saturation values of BSA and Fg for a range of sized hydrophilic and hydrophobic spheres. Error bars show 95% confidence limits.

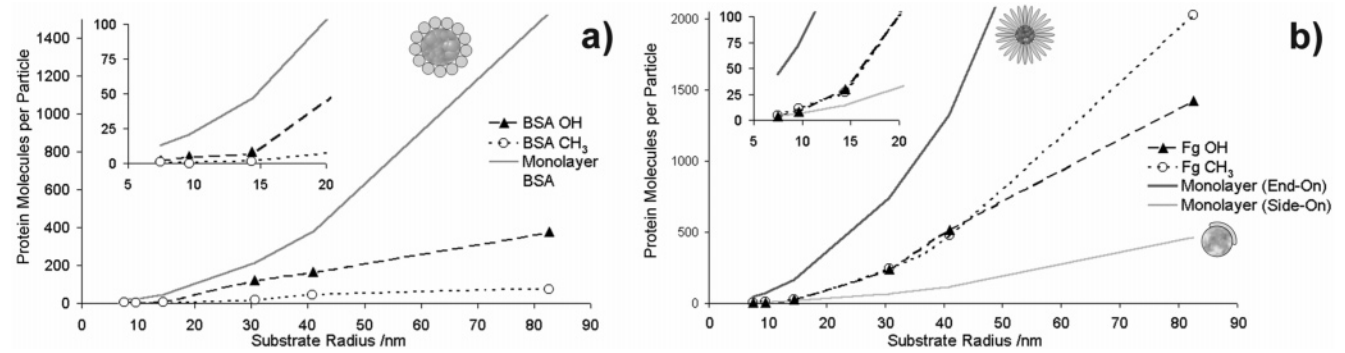

Figure 4. Saturation values obtained for (a) BSA and (b) fibrinogen onto −▲− hydrophilic and −○− hydrophobic surfaces tested. Inserts show schematic models of surface-bound proteins adjacent to corresponding monolayer curves.

hydrophobic surfaces in the range tested, but increases as particle size increases due to the greater surface area available. It should be noted that a close-packed monolayer was not reached during this investigation, possibly suggesting that albumin denaturation hinders such a packing arrangement (see below).

Fibrinogen follows the trend observed for albumin, with higher affinity constants and lower saturation values when adsorbing onto smaller particles. Due to the shape of fibrinogen, two possible adsorption orientations can be achieved: side-on, with its long axis perpendicular to the radius, or end-on, with its long axis parallel to the radius.[8] Interestingly, for particles having radii less than ∼20 nm, the amount adsorbed corresponds well to that expected for a side-on monolayer. Above this sphere size a side-on monolayer is exceeded, although an end-on monolayer is not reached, possibly due to the constraints of close packing. Fibrinogen adsorption is therefore controlled to a large extent by surface curvature on the nanoscale, although with respect to surface chemistry little difference was observed, the protein adsorbing in similar amounts to both hydrophilic and hydrophobic surfaces tested.

Conformational Assessment. Infrared spectroscopy using an attenuated total reflectance (ATR) accessory has been used for the analysis of adsorbed protein conformation. Protein infrared spectra contain peaks arising mainly from amide bond vibrations. The amide I band centered at ∼1700−1600 cm^{-1} is largely due to C═O stretching vibrations. This band is sensitive to changes in secondary structure and has therefore been widely used for protein conformational studies.[8,18,23−25] Amide bonds within differing secondary structures give rise to specific vibrational bands. These component bands are largely overlapping and all

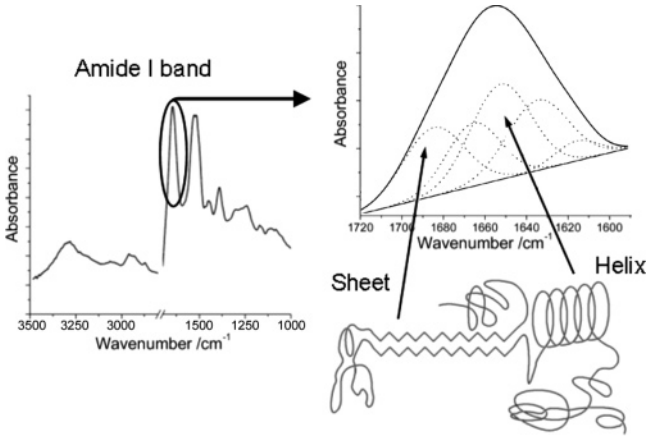

Figure 5. Example infrared spectra for BSA adsorbed onto 30 nm silica particles with CH_3 terminal chemistry. Dotted lines show fitted component peaks.

contribute to the characteristic broad amide I band observed in IR spectra. The overall shape of the band is determined by the various secondary structure components of the protein analyzed, for example, α-helices, β-sheet, β-turn, and unordered structures, Figure 5, (other spectra can be found in the Supporting Information).[8,18,23−25] From previous studies, component bands with maxima at 1685−1663 cm^{-1} have been assigned to β-sheet or β-turn structures, at 1655−1650 cm^{-1} to α-helices, at 1648−1644 cm^{-1} to random chains, at 1639−1635 cm^{-1} to extended chains, and at 1632−1621 cm^{-1} to extended chains or β-sheets. Bands observed at lower wavenumbers, ∼1610 cm^{-1}, are often considered to arise from intermolecular bonding.[23−25]

Albumin has a less organized secondary structure when

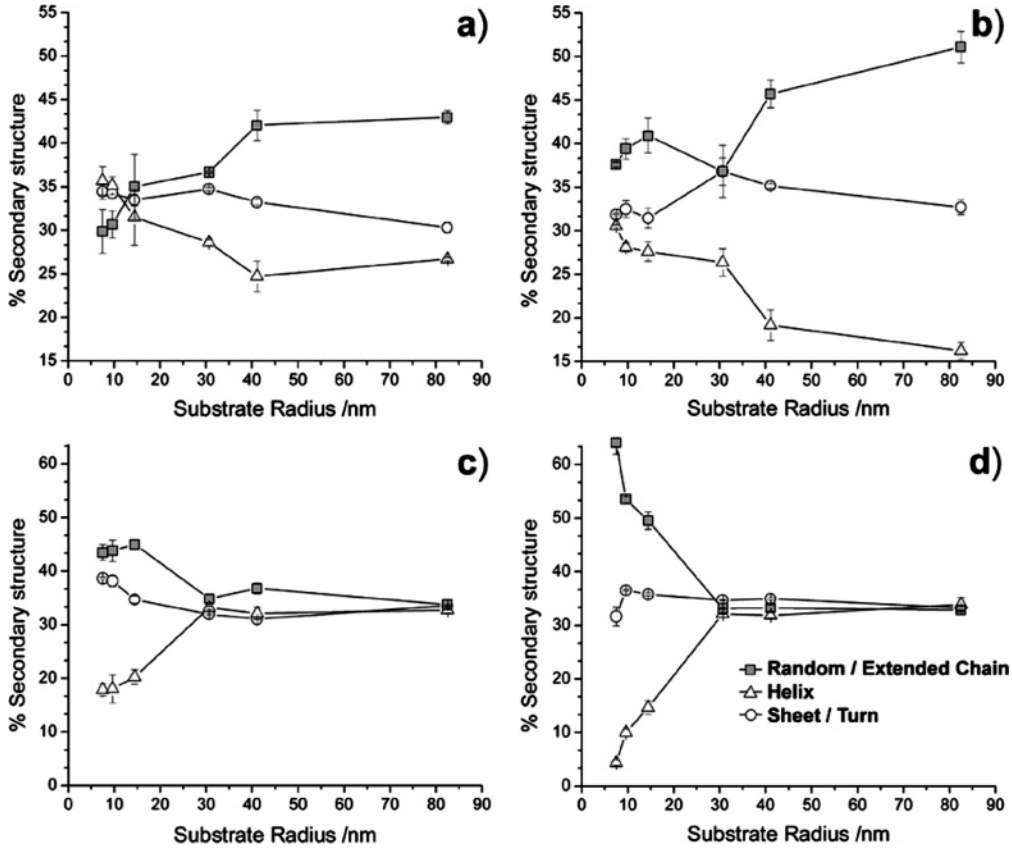

Figure 6. Conformational assessment of surface-bound BSA (a) and (b); fibrinogen (c) and (d) onto hydrophilic (a) and (c); and hydrophobic (b) and (d) substrates. Random coil/extended chain structure −□−; α-helix −△−; β-sheet/β-turn −○−.

adsorbed onto larger spheres, predominantly losing helical structure with a corresponding increase in random coil/extended chain structure. A large change in secondary structure is observed for albumin adsorbed onto substrates with radii between ∼10 and 40 nm, with a rise in random structure of about 15% and a 10% loss of helical component on hydrophilic surfaces. Loss of ordered structure is not linear with substrate size, becoming less apparent on particles with radii above ∼40 nm, Figure 6a. Albumin adsorbed onto substrates with high surface curvature retains more nativelike structure. Such trends are observed for both hydrophilic and hydrophobic surfaces, although a greater loss of structure is observed on the latter with ∼15% loss of helical structure, Figure 6b.

In contrast, fibrinogen becomes more disordered on smaller spheres, losing ∼12% helical structure at the expense of an increasing random chain component on hydrophilic surfaces, Figure 6c. Onto spheres with radii above 30 nm, little variation in fibrinogen conformation is seen. This trend is observed for both hydrophilic and hydrophobic surfaces, but again is more pronounced on the latter, where almost all (30%) helical structure is converted to random coil, Figure 6d.

Similar to the amide I band, the amide II band centered at around 1500−1600 cm^{-1} is composed of component in-plane N−H bending and C−N stretching bands of the amide bond. Due to the lower intensity and different vibrational modes contained, this band is less sensitive to conformational changes compared to the amide I region. The amide I/II ratio has, however, been used to qualitatively assess conformation/orientation changes.[8,18] Our data suggest subtle differences in the orientation of albumin and fibrinogen when adsorbed onto differently sized spheres. Although such a ratio can give little qualitative information, it is interesting to observe variation in bound protein structure and one can use this as a secondary measure of conformation/orientation change. Generally higher amide I/II ratios are observed for albumin and fibrinogen with increasing substrate size, Figure 7. This indicates that both proteins bind, having different geometries onto substrates with differing surface curvature. These data show significantly different amide I/II ratios (both height and area ratios) for proteins adsorbed onto particles above ∼30 nm radius compared to proteins adsorbed on smaller particles. This corresponds well with the amide I curve-fitting analysis, again suggesting that both albumin and fibrinogen have differing conformations and/or orientations when adsorbing onto small particles with radii <30 nm than when adsorbed onto larger particles. Band ratios for albumin are greater than those observed for fibrinogen, which may indicate a greater degree of structural change.

Protein−Protein Interactions. The extent of intermolecular bonding can be used as an indication of lateral protein−protein interactions experienced by neighboring bound proteins. It has been suggested by others that amide I component bands centered at ∼1610 cm^{-1} are sensitive to hydrogen bonding and can be used as a measure of intermolecular protein−protein interaction.[23−25] Analysis of the contribution of this peak to the total amide I area suggests that the interaction between bound proteins (both BSA and Fg) is generally greater on hydrophilic compared to hydrophobic surfaces, Figure 8. This is probably an effect ofsurface-induced deformation as spreading of the protein would reduce the interaction area between neighboring adsorbed proteins. Fibrinogen, however, again shows differing charac-

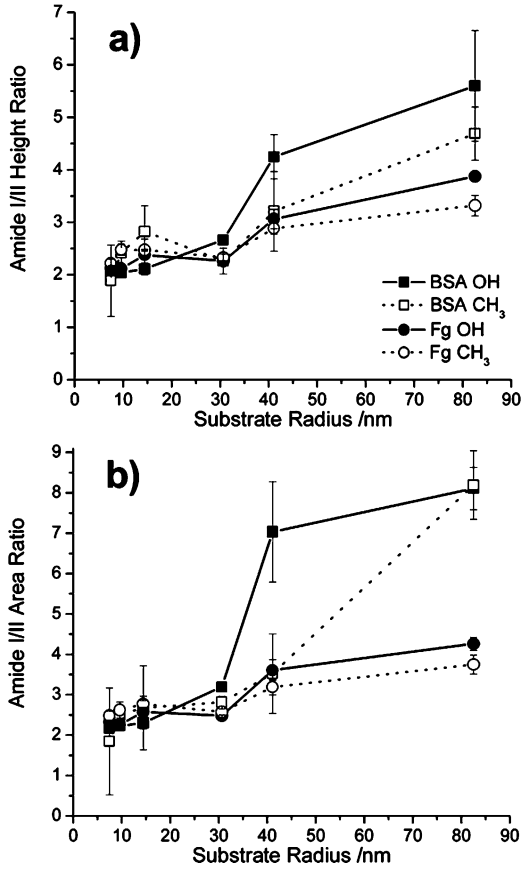

Figure 7. Variation in amide I/II, (a) height and (b) area ratio. Error bars show standard deviation of triplicate samples.

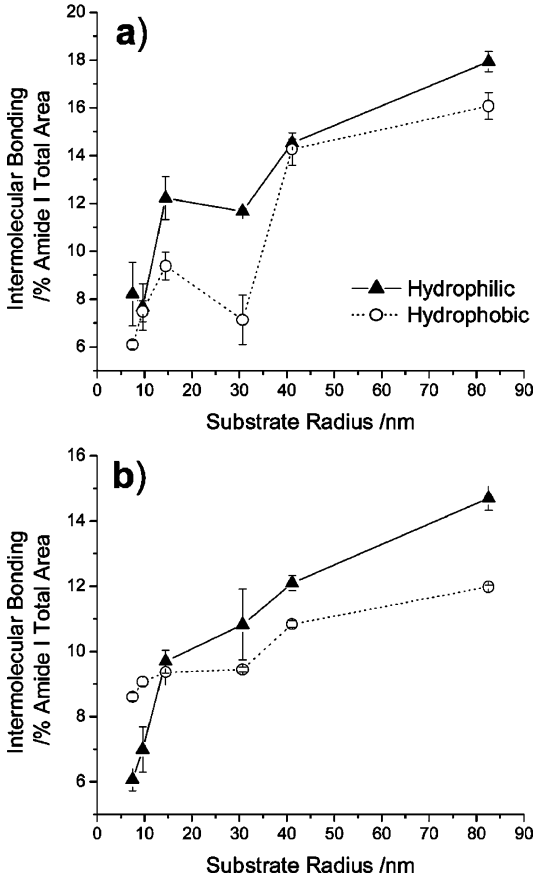

Figure 8. Bound protein−protein interactions of (a) BSA and (b) Fg on −▲− hydrophilic and −○− hydrophobic surfaces.

teristics on spheres with radii <30 nm, where intramolecular bonding becomes higher for hydrophobic substrates. A trend toward higher intramolecular interaction with increasing sphere size suggests greater lateral interaction between bound proteins, which is expected from the geometry of adjacent protein molecules.

Discussion. Protein surface interactions can induce conformational changes in the adsorbing protein molecules. It has been reported previously that both BSA and Fg undergo greater conformational disordering when adsorbing onto hydrophobic rather than hydrophilic surfaces, a finding supported by this study. However, few studies have been conducted that examine the effects of topography on protein adhesion. Here we describe protein−surface interactions of albumin and fibrinogen using surface curvature as a measure of topography.

Albumin and fibrinogen interact very differently in relation to changes in surface curvature. Although research into the effects of surface topography on protein adsorption is in its infancy, an investigation examining lysosyme adsorption, a small globular protein comparable in size to albumin, strongly suggests that substrate curvature modifies protein conformation upon binding.[13] Few differing particle sizes were analyzed and structural detail of the adsorbed species was limited such that only protein molecules adsorbed onto the smaller particles were able to be investigated. Furthermore, previous investigations have concentrated on small globular proteins.[9,13,15] To our knowledge there has been no systematic evaluation of protein responses to surfaces, taking into account protein size and shape as well as surface curvature and chemistry. Here we extend the knowledge base of protein−surface interactions by examining

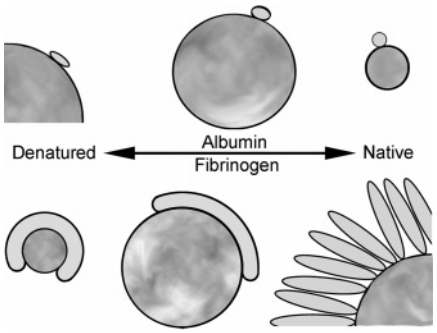

Figure 9. Schematic demonstrating control of conformation and orientation by surface curvature.

the above effects on binding and structural characteristics of two geometrically very different proteins. In addition, a number of particle sizes were investigated, ranging from those presenting high surface curvature to those for which the protein molecules would observe a pseudo-flat surface.

Particles with high surface curvature were found to stabilize the nativelike conformation of albumin, Figure 9, thus supporting the earlier results on lysosyme adsorption.[13] The same effect for variants of human carbonic anhydrase, other globular proteins of comparable size to albumin, have also been reported.[14,33] We have also shown that the effects of surface curvature are also compounded by variation in surface chemistry. Albumin becomes more disordered upon binding to hydrophobic surfaces. Such disorder is more pronounced on particles with

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radius larger than ~30 nm. Amide I/II ratios also show a change above 30 nm, demonstrating a difference in orientation/conformation of albumin bound on particles above and below this size.

Fibrinogen, in sharp contrast to albumin, becomes more denatured upon binding to smaller particles, i.e., those having higher surface curvature. A previous study has suggested that fibrinogen, having a rodlike shape, can adsorb in two possible orientations: side-on, with its long axis parallel to the particle radius, or end-on, with its long axis perpendicular to the radius.[8] The current study demonstrates that side-on orientation is favored on smaller particles, Figure 4. A large change in fibrinogen conformation was observed on adsorption to particles with radii less than 30 nm ($^3/_5$ of the length of fibrinogen). Upon adsorption the molecule possibly wraps around the surface. Protein structural perturbation would therefore be expected to be greater on small particles with higher curvature, as was observed with the loss of the helical component. Such conformational change would be encouraged by an increase in protein−surface interaction that would probably also hinder surface rearrangement of the protein to the end-on orientation. With adsorption onto larger particles, surface curvature may be too small to induce such structural alteration. An end-on orientation could then be reached, driven by increased protein−protein interactions. This hypothesis is supported by the increased amide I/II ratios and observed intermolecular bonding on particles with radii $\geq$30 nm. Furthermore, if rodlike proteins were to adsorb onto highly curved surfaces in an end-on orientation, their backbones would be separated and neighboring proteins would interact with each other to minimize solvation by water around their hydrophobic domains. Therefore, onto highly curved surfaces an end-on orientation of fibrinogen is not feasible. The effects of surface curvature on fibrinogen conformation are emphasized by surface chemistry, a finding also observed for albumin. Fibrinogen has a greater degree of random coil/extended chain structure on hydrophobic surfaces, as does albumin, suggesting that hydrophobic protein−surface interactions govern the structure of surface-bound protein.

Speculative Discussion. The conformation of adsorbing proteins can change considerably upon binding to surfaces having differing chemistries. Furthermore, the shape of proteins dictates the possible orientations of the molecules once on the surface. Topography at the nanoscale must be an important factor determining how proteins "observe" the surface. Using surface curvature as a simple model of topography, we have shown that proteins interact with surface features. More importantly, we have shown that proteins of differing shapes can associate with a surface in quite dissimilar ways with fibrinogen being denatured to a greater extent on highly curved surfaces in contrast to albumin. From the results presented it is clear that the protein−surface binding affinity and packing density of adsorbed proteins is also dependent upon surface chemistry and curvature.

By designing surfaces with particular nanoscale topography and defined surface chemistry, it may be possible to tailor a materials performance upon implantation into a host. For example, using fibrinogen and albumin as simple examples, it may be possible to design specific surfaces to allow fibrinogen to dominate at a surface, such that platelet/cell adhesion occurs and a material/implant rapidly becomes integrated into the host. Conversely, if the surface was designed to allow albumin to adsorb preferentially, accumulation of biomatter would be obstructed. These two extreme cases could be employed to aid integration of implants according to their intended usage; e.g., for material to be useful as a hip replacement, the surface should be designed to promote formation of a protein layer comprised mainly of cell adhesive proteins such as fibrinogen and fibronectin with these proteins being presented in the correct orientation and conformation to mediate cell attachment. Other implants which are required to resist biomatter adhesion such as vascular stents would benefit from an anti-biofouling coating which either resists protein adhesion altogether or allows nonadhesive proteins such as albumin to adhere that block cell adhesion.

Conclusions

Protein−surface interactions play an important role in determining the adsorbed conformation and, therefore, activity of surface-bound proteins/enzymes. Here we describe the effects of surface curvature, with and without surface chemistry. Albumin and fibrinogen have dramatically differing interactions largely due to their differing shapes. Trends in binding affinity and surface saturation amounts correspond well with the extent of conformational change of the bound proteins. Together these data describe how the structure of albumin, a globular protein, is stabilized by high surface curvature. On the other hand, fibrinogen, a rodlike protein, is distorted by wrapping around surface curvature, inducing secondary structure loss.

With understanding how biomolecules are affected by the nature of surfaces, control of their bound states can be achieved. This may give rise to an array of new tailor-made materials/surface coatings for use as biosensors, hybrid materials, nanotechnology, and biocompatible surfaces. Of particular current interest is the control over enzymatic activity or cell adhesion that can be harnessed by fine-tuning surface coatings.

Acknowledgment. We gratefully acknowledge funding from Smith and Nephew and would like to thank Mr. D. Belton for carrying out nitrogen adsorption analysis and Dr. N. J. Shirtcliffe and Dr. S. V. Patwardhan for their useful discussions.

Supporting Information Available: Infrared analysis of component amide I bands for all data sets (albumin and fibrinogen adsorbed onto hydrophilic and hydrophobic spheres of all sizes) and UV−Vis scans of albumin and fibrinogen. This material is available free of charge via the Internet at http://pubs.acs.org. See any current masthead page for ordering information and Web access instructions.

JA056278E