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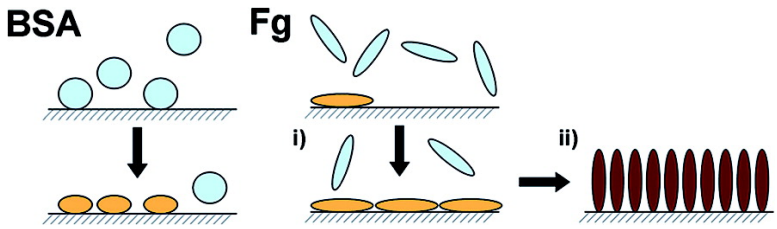

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Interpretation of Protein Adsorption: Surface-Induced Conformational Changes

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Abstract: Protein adhesion plays a major role in determining the biocompatibility of materials. The first stage of implant integration is the adhesion of protein followed by cell attachment. Surface modification of implants (surface chemistry and topography) to induce and control protein and cell adhesion is currently of great interest. This communication presents data on protein adsorption (bovine serum albumin and fibrinogen) onto model hydrophobic (CH_3) and hydrophilic (OH) surfaces, investigated using a quartz crystal microbalance (QCM) and grazing angle infrared spectroscopy. Our data suggest that albumin undergoes adsorption via a single step whereas fibrinogen adsorption is a more complex, multistage process. Albumin has a stronger affinity toward the CH_3 compared to OH terminated surface. In contrast, fibrinogen adheres more rapidly to both surfaces, having a slightly higher affinity toward the hydrophobic surface. Conformational assessment of the adsorbed proteins by grazing angle infrared spectroscopy (GA-FTIR) shows that after an initial 1 h incubation few further time-dependent changes are observed. Both proteins exhibited a less organized secondary structure upon adsorption onto a hydrophobic surface than onto a hydrophilic surface, with the effect observed greatest for albumin. This study demonstrates the ability of simple tailor-made monochemical surfaces to influence binding rates and conformation of bound proteins through protein−surface interactions. Current interest in biocompatible materials has focused on surface modifications to induce rapid healing, both of implants and for wound care products. This effect may also be of significance at the next stage of implant integration, as cell adhesion occurs through the surface protein layer.

Introduction

Biocompatibility is closely related to cell behavior on contact with implant surfaces[1] and is decided by two main factors: the response of the host to the implant and the response of the implant in the host. It has been suggested that biological tissues interact with only the outermost atomic layers of an implant[1,2] and consequently much research has been devoted to methods that modify the surfaces of existing biomaterials in order to achieve more desirable biological integration. Changes in morphological, physicochemical, and biochemical aspects of biomaterials have been investigated.[2]

Cell adhesion is mediated by interaction with preadsorbed proteins during implant integration. The adsorption of proteins from blood to biomaterial surfaces is a dynamic process during which proteins may bind, rearrange, and detach.[1] Thus, the biomaterial surface can be coated with a range of proteins long before any interaction occurs with platelets or cells. Although it is now generally accepted that the initial stages of protein adsorption to biomaterials plays an important role in the incorporation of the implant,[1] earlier research in this field mainly focused on cell-surface interactions.[3−6] A number of factors including surface chemistry, charge, topography, and wetting behavior have been shown to play a role in determining cell growth and proliferation.

Proteins adsorb in differing quantities, densities, conformations, and orientations, depending on the chemical and physical characteristics of the surface.[7−11] Protein adsorption is a complex process involving van der Waals, hydrophobic and electrostatic interactions, and hydrogen bonding. Although surface−protein interactions are not well understood, surface chemistry has been shown to play a fundamental role in protein adsorption.[8,9,11] Moreover, the properties of protein over-layers can be altered by the underlying chemistry, which directly impinges on control of conformation and/or orientation.[8,9] Adsorbed proteins may

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(1) Kasemo, B. *Adv. Dental Res.* **1999**, *13*, 1−20.
(2) Puleo, D. A.; Nanci, A. *Biomaterials* **1999**, *20*, 2311−2321.
(3) Anselme, K. *Biomaterials* **2000**, *21*, 667−681.
(4) Chesmel, K. D.; Clark, C. C.; Brighton, C. T.; Black, J. *J. Biomed. Mater. Res.* **1995**, *29* (9), 1101−1110.
(5) Fredriksson, C.; Khilman, S.; Kasemo, B.; Steel, D. M. *J. Mater. Res.: Mat. Res.* **1998**, *9* (12), 785−788.
(6) Lampin, M.; Warocquier-Clerout, R.; Legris, C.; Degrange, M.; Sigot-Luizard, M. F. *J. Biomed. Mater. Res.* **1997**, *36* (1), 99−108.
(7) Pitt, W. G.; Cooper, S. L. *J. Biomed. Mater. Res.* **1988**, *22*, 359−382.
(8) Taborelli, M.; Eng., L.; Descouts, P.; Ranieri, J. P.; Bellamkonda, R.; Aebischer, P. *J. Biomed. Mater. Res.* **1995**, *29* (6), 707−714.
(9) Lenk, T. J.; Horbett, T. A.; Ratner, B. D.; Chittur, K. K. *Langmuir* **1991**, *7* (8), 1755−1764.
(10) Sigal, G. B.; Mrksich, M.; Whitesides, G. M. *J. Am. Chem. Soc.* **1998**, *120* (14), 3464−3473.
(11) Ta, T. C.; McDermott, M. T. *Anal. Chem.* **2000**, *72*, 2627−2634.

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act as pilots for cell adhesion if they have the correct geometry to mediate cell attachment. For example, integrin binding sites of cells interact with specific peptide regions within proteins (e.g. the RGD tripeptide in fibronectin[12]). If these are exposed and available, then attachment may occur.

Although cell-surface interactions are understood to be affected by underlying surface chemistry, structural information on surface bound protein conformations and geometries governing cell adhesion have not yet been elucidated. The ability of antibody binding assays to detect the availability of specific binding sites provides indirect evidence of protein conformational or orientational change upon adsorption, although no structural information can be obtained from such investigations.[13] Other analytical tools have been used to study protein adsorption.[8,13−19] These methods yield only generalized information on the adsorbed protein implying conformational changes; however, no quantitative data on the secondary structure of the adsorbed protein has been presented.

During the past decade substantial progress has been made in understanding the mechanism of protein adsorption. Researchers have developed a number of techniques, e.g. QCM,[14−16] surface plasmon resonance (SPR),[15−17] ellipsometry,[15] FTIR,[19] atomic force microscopy (AFM),[8,11,16,18] to tackle this problem although none alone are able to monitor the whole process of adsorption, rearrangement, and possible desorption. Here we describe the use of a quartz crystal microbalalance (QCM) and grazing angle Fourier transform infrared spectroscopy (GA-FTIR) to obtain information on the rate and amounts of protein adsorbed as well as on the conformation of the surface-bound protein. To our knowledge this is the first report illustrating the use of QCM together with GA-FTIR to assess protein−surface interactions. QCM allows an insight into the rate and amounts of protein adsorbed while FTIR allows the assessment of protein conformation which has previously been well documented.[9,19−21]

We describe the adsorption characteristics of two plasma proteins: bovine serum albumin (BSA), the most abundant plasma protein which transports fatty acids along with other small molecules throughout the circulatory system and bovine fibrinogen (Fg), the most abundant protein involved in the coagulation cascade.

It is important to understand the effects of different surface properties on protein adhesion, i.e., chemistry and topography. Here we focus on the influence of surface chemistry. The two surfaces examined exhibit differing wetting behavior due only to their contrasting chemically defined surfaces. Surfaces under investigation were first produced via self-assembly of alkane- and alcohol-containing thiols, affording model hydrophobic and hydrophilic surfaces, respectively.

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 phosphate) and NaCl (100 mmol) obtained from Aldrich to give pH 7.4 at 25 °C. Protein solutions with concentrations in the range 50−1000 μg mL^{-1} were prepared immediately before use. Distilled deionized water with a conductivity of ∼1 μS was used for all experiments.

Preparation of Gold-Coated Surfaces. Substrates were prepared by sputter deposition of 3 nm titanium and 30 nm gold onto glass microscope slides. Sputter coating was conducted using an Emitech K575. The surfaces were washed in ethanol, dried under nitrogen, and stored in a desiccator before use. QCM crystals with Cr/Au electrodes (Testbourne LTD) were cleaned before use using piranha etch, 1:3 H_2O_2:H_2SO_4 (Fisher Chemicals and Sigma, respectively), rinsing with distilled, deionized water, and ethanol before use.

Preparation of SAMs on Gold-Coated Surfaces and QCM Crystals. Chemically defined surfaces were prepared by self-assembled monolayers (SAMs) of thiols: heptanethiol and mercaptoethanol (Sigma) to give hydrophobic and hydrophilic surfaces, respectively. Gold-coated microscope slides and QCM crystals were incubated in 1 mM ethanolic solutions of the desired thiol for at least 12 h before use, rinsed with ethanol, and dried under nitrogen. Surface wettability was characterized by taking images of 5 μL water droplet equilibrium contact angles on a Krüss DSA10. Droplets were applied by a microsyringe with a hydrophobized needle, and images were taken immediately to eliminate drying effects.

Surfaces prepared using heptanethiol and mercaptoethanol were used as model hydrophobic and hydrophilic surfaces. The use of SAMs has been reported elsewhere[22] and has been widely used for the preparation of chemically defined surfaces. The driving force for monolayer formation is the maximization of strong gold−sulfur bonds and chain−chain hydrophobic interactions, which results in a densely packed, crystalline monolayer. Heptanethiol-treated surfaces showed a contact angle of 94°, whereas on mercaptoethanol-treated surfaces the contact angle was 48°.

Quartz Crystal Microbalance (QCM). Quartz crystal microbalance measurements were made using a Maxtek PLO10 oscillator. Dual piston liquid pumps were used to linearly flow solutions at a rate of three volume changes per minute of the FC-550 flow cell over a 25 mm diameter 5 MHz crystal. PBS served as a background after which the protein solution was introduced at the same flow rate.

Upon protein adsorption to the crystal surface, the oscillatory motion of the crystal was dampened, causing a decrease in the resonant frequency. The frequency shift of the QCM is due to a change in total coupled mass, including water interaction within the protein layer as for this study. The Sauerbrey equation relates the measured frequency shift (Δf) and the adsorbed mass per unit area (M).[23]

$$M = -C\frac{\Delta f}{n}$$

where C = mass sensitivity constant (17.7 ng cm^{-2} Hz^{-1} for 5 MHz crystals), n = overtone number; 1, 3...n.

The proportionally between these two variables is not *strictly* true for this system, due to the viscoelastic contribution of the protein adlayer and hydration effects. Deviation from this relationship when investigating non-rigid adlayers does not allow direct calculation of mass-loading,

(12) Johansson, S.; Svineng, G.; Wennerberg, K.; Armulik, A.; Lohikangas, L. *Front. Biosci.* **1997**, *2*, 126−146.
(13) Kiaei, D.; Hoffman, A. S.; Horbett, T. A.; Lew, K. R. *J. Biomed. Mater. Res.* **1995**, *29* (6), 729−39.
(14) Höök, F.; Rodahl, M.; Brzezinski, P.; Kasemo, B. *Langmuir* **1998**, *14*, 729−734.
(15) Höök, F.; Kasemo, B. *Anal. Chem.* **2001**, *73*, 5796−5804.
(16) Caruso, F.; Furlong, D. N.; Kingshott, P. *J. Colloid Interface Sci.* **1997**, *186*, 129−140.
(17) Sigal, G. B.; Mrksich, M.; Whitesides, G. M. *J. Am. Chem. Soc.* **1998**, *120* (14), 3464−3473.
(18) Kidoaki, S.; Matsuda, T. *Langmuir* **1999**, *15*, 7639−7646.
(19) Chittur, K. K. *Biomaterials* **1998**, *19* (4−5), 357−369.
(20) Wu, Y.; Murayama, K.; Czarnik-Matusewicz, B.; Ozaki, Y. *Appl. Spectrosc.* **2002**, *56* (9), 1186−1193.
(21) Fu, F.-N.; DeOliveira, D. B.; Trumble, W. R.; Sarkar, H. K. *Appl. Spectrosc.* **1994**, *48* (11), 1432−1441.
(22) Ferretti, S.; Paynter, S.; Russell, D. A.; Sapsford, K. E.; Richardson, D. J. *Trends Anal. Chem.* **2000**, *19* (9), 530−540.
(23) Sauerbrey, G. *Z. Phys.* **1959**, *155*, 206−22.

although it can be used as an indication of mass if it is understood *only* to be an estimation.[15,24]

Grazing Angle FTIR (GA-FTIR). Infrared analysis of surface-bound protein was conducted using an FT-80 grazing angle accessory (Thermo Nicolet) in a Magna IR-750 spectrometer, continuously purged with dry air/nitrogen. A polarizer, set to allow only p-polarized light to pass through the sample, was used to obtain enhanced spectra. Ti/Au-coated slides were incubated in protein solutions, removed after a time, rinsed in distilled, deionized water, and dried under a stream of nitrogen before immediate examination. Spectra were recorded at 4 cm^{-1} resolution with 512 scans being averaged, smoothed by 9 pt adjacent averaging, and curve-fitted.

The fitting procedure was such that the amide I band was treated, having a linear baseline between 1720 and 1580 cm^{-1}. Component peaks were fitted with Gaussian band profiles. Peak positions were estimated from literature[9,19−21] although not fixed during fitting.

Results and Discussion

Proteins under Investigation: Bovine Serum Albumin (BSA) and Fibrinogen (Fg). Proteins with significant differences in their size and shape were chosen for investigation. With a molecular weight of 66 kDa, albumin is the most abundant protein in the circulatory system. Acting as a multifunctional transporter protein, it has a concentration of approximately 50 mg mL^{-1} plasma. Early research led to the belief that serum albumin had an ellipsoid shape with dimensions of 14 by 4 nm although ^1H NMR studies indicate a heart-shaped structure[25] in good agreement with X-ray crystallographic data.[26] Data on the secondary structure of albumin is somewhat contradictory with some researchers suggesting predominantly α-helical (50−68%) structure with the remaining being made up of β-sheet (16−18%)[27−29] but with other research suggesting that the protein polypeptide contained no β-sheet structure, rather having turns and extended flexible regions instead.[30]

Fibrinogen, found in the circulatory system at a concentration of 2.6 mg mL^{-1} is the key structural glycoprotein in blood clotting which, upon thrombin activation, self-assembles forming a fibrin clot. Having a mass of 340 kDa, the elongated molecule, 46 nm long,[31] is a genuine covalent dimer, the two halves having identical sequences that are linked by a central globular domain. Each monomer has three non-identical chains, Aα, Bβ, and γ, connected together at the N-terminus by 11 disulfide bridges forming the 'disulfide knot'. The C-terminus of each chain is globular. Those of the β and γ chains extend forming dumbbell shaped ends to the molecule termed the 'D' regions, while the Aα chain globular domains, termed the αC units, interact with each other at the central 'E' region.

Quartz Crystal Microbalance Measurements. When a liquid is placed on a surface, it may spread out or sit as a droplet on the surface. The behavior depends on the relative interfacial energies of the liquid and the solid surface. The ability of a droplet to spread can be assessed by the contact angle of a droplet, described as the angle between the water−solid interface and the water−air interface.[32] Water contact angles are used to assess surface wettability. The two surfaces used in this study were chosen for their contrasting chemistries, giving water contact angles of 94° and 48° for CH_3 and OH terminated surfaces, respectively. As an example of the data obtained, Figure 1 shows the QCM adsorption profiles of fibrinogen onto a hydrophobic alkane terminated surface.

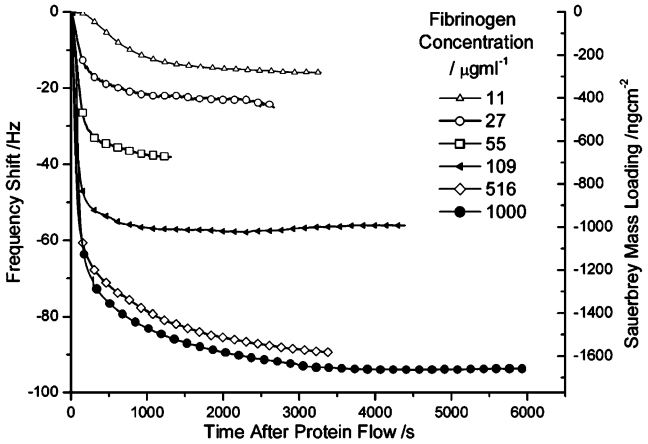

Figure 1. Fibrinogen adsorption profiles onto CH_3-terminated surfaces.

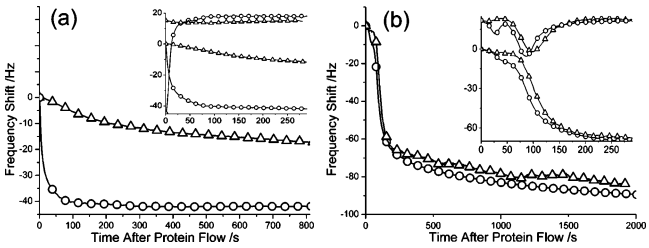

Figure 2. Adsorption profiles of (a) BSA and (b) fibrinogen onto CH_3 (O) and OH (Δ) terminated surfaces. Initial bulk protein concentration was 1 mg mL^{-1}. Inserts show derivative plots with corresponding initial adsorption profiles.

With increasing protein concentration, a greater frequency shift and higher rate of frequency change was observed for adsorption onto both CH_3- and OH-terminated surfaces. Although this trend is true for both fibrinogen and BSA, differing protein−surface interactions are apparent from the shapes of the adsorption profiles. At higher concentrations the equilibrium protein−surface concentration increases to a limiting value due to surface saturation. At lower concentrations it is expected that protein molecules will adsorb until an equilibrium surface concentration is reached. At this point the amount of protein adsorbing is equal to that desorbing from the surface. The rate of adsorption of BSA onto the CH_3-terminated surface is greater than that onto the OH-terminated surface tested, whereas Fg seems to undergo a similarly rapid adsorption process on both, Figure 2.

The data shows that BSA has a weaker attraction toward the hydrophilic than the hydrophobic surface. A convenient method to assess the protein−surface affinity is to deduce 'binding constants' that compare the rate of adsorption to desorption. When data is fitted to the Langmuir isotherm, the binding constant K and a saturation value for the amount adsorbed can be estimated. K relates to the affinity of the adsorbent to the

(24) Voinova, M. V.; Jonson, M.; Kasemo, B. *Biosens. Bioelectron.* **2002**, *17*, 835−841.
(25) Bos Labro, O. J. M.; Jan, F. A.; Fischer, M. J. E.; Wilting, J.; Janssen, L. H. M. *J. Biol. Chem.* **1989**, *264* (2), 953−9.
(26) Carter, D. C.; He, X. M.; Munson, S. H.; Twigg, P. D.; Gernert, K. M.; Broom, M. B.; Miller, T. Y. *Science* **1989**, *244*, 4909, 1195−8.
(27) Sjoholm, I.; Ljungstedt, I. *J. Biol. Chem.* **1973**, *248* (24), 8434−41.
(28) Reed, R. G.; Feldhoff, R. C.; Clute, O. L.; Peters, T., Jr. *Biochemistry* **1975**, *14* (21), 4578−83.
(29) Foster, J. F. *Albumin Struct., Funct. Uses* **1977**, 53−84.
(30) Carter, D. C.; Ho, J. X. *Adv. Protein Chem.* **1994**, *45*, 153−203.
(31) Yang, Z.; Mochalkin, I.; Veerapandian, L.; Riley, M.; Doolittle, R. F.; *Biochemistry* **2000**, *97* (8), 3907−3912.
(32) Shirtcliffe, N. J.; Aqil, S.; Evans, C.; McHale, G.; Newton, M. I.; Perry, C. C.; Roach, P. *J. Micromech. Microeng.* **2004**, *14*, 1384−1389.

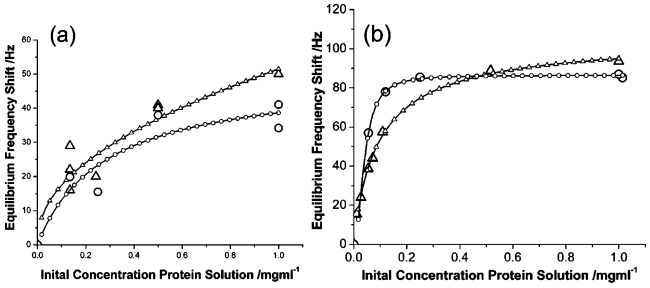

Figure 3. Langmuir adsorption isotherms of (a) BSA and (b) fibrinogen onto CH_3- (O) and OH- (Δ) terminated surfaces.

Table 1. Binding Constants and Saturation Values for BSA and Fibrinogen

	binding constant	saturation values, Hz
BSA **OH**[a]	5.3	47.2
BSA $\mathbf{CH_3}$[a]	5.4	40.9
Fg **OH**	10.9	102.0
Fg $\mathbf{CH_3}$	36.0	92.6

[a] **OH** and $\mathbf{CH_3}$ represent hydrophilic and hydrophobic surfaces, respectively.

adsorbate with a high value representing higher affinity. Langmuir isotherms for BSA and Fg onto CH_3- and OH-terminated surfaces are shown in Figure 3, and values (Table 1) of K were calculated as 5.3 and 5.4 for BSA onto OH- and CH_3-terminated surfaces, respectively. Surface saturation frequency shift values of 47.2 and 40.9 Hz were also obtained for BSA adsorption onto the hydrophilic and hydrophobic surfaces, respectively.

Greater amounts of BSA can adsorb to a hydrophilic surface, although higher affinity toward the hydrophobic surface causes deformation of the protein (see later), and this helps to explain the lower surface coverage at saturation. Similarly, Fg has a greater affinity toward the CH_3-terminated surface ($K = 36.0$ versus 10.9 for the OH surface) and has lower saturation values (92.6 versus 102.0 Hz). The greater frequency shift due to greater mass loading was as expected for the higher molecular weight fibrinogen.

The adsorption profiles of both proteins on the surfaces tested, Figure 2, indicate that for albumin onto alkane-terminated surfaces and fibrinogen onto both surfaces adsorption is rapid, slowing as surface coverage increases. Proteins adsorbing later in the process compete for free sites that become fewer as coverage increases. The adsorption process for fibrinogen is complex and is clearly visible in the derivatives of the adsorption profiles (Figure 2 inserts). The adsorption process appears to occur in a stepwise fashion with initial rapid adsorption and sequential slowing ($\sim$50 s), followed by a second rapid adsorption stage, again progressively slowing but over a longer period ($\sim$60 min). The results may indicate that the protein initially adsorbs rapidly with its long axis parallel to the surface, thus covering the surface quickly. After this time, rearrangement of the protein to perhaps a perpendicular orientation may allow further protein molecules to adsorb on the uncovered, free sites, a process that is observed as a second rapid adsorption stage. A schematic of possible protein adsorption processes is shown in Figure 4.

Multistage adsorption of fibrinogen was only observed at high concentrations, supporting the proposal for molecular rearrangement of the protein on the surface. Such surface rearrangement

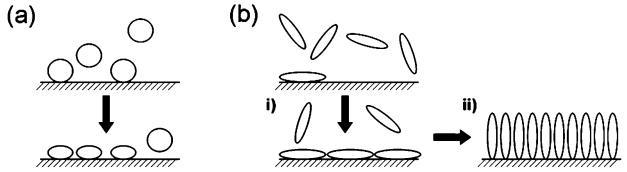

Figure 4. A schematic to show adsorption of (a) a globular protein (e.g. BSA) whose conformation may become distorted on interaction with the surface and (b) a rod-like protein that undergoes a multistage adsorption process where (i) initially the protein adsorbs with its long axis parallel to the surface and then (ii) rearrangement occurs to increase protein−protein interaction and surface concentration of protein.

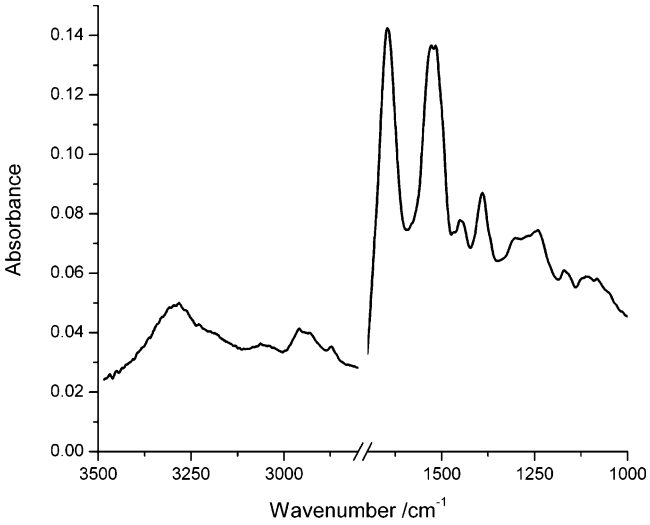

Figure 5. A sample infrared spectrum of BSA.

may be driven by increased hydrophobic interaction of the fibrinogen molecules as their long axes become aligned parallel to each-other. In contrast, albumin seems to undergo a single-step adsorption prpcess at the concentrations used. Albumin is approximately globular in shape compared to fibrinogen, so adsorption in any orientation would result in almost the same surface area coverage and packing, Figure 4.

It should be noted that the initial adsorption of fibrinogen appears to be affected by the underlying surface chemistry. Derivatives of adsorption profiles show that the first adsorption stage is faster and well defined onto a CH_3-terminated surface, but the second adsorption stage reaches a maximum earlier in time compared with adsorption onto an OH-terminated surface. These data suggest that there is a greater attractive force between a fibrinogen molecule and a hydrophobic surface. Albumin adsorbs more rapidly to the CH_3 surface than onto the OH-terminated surface, which may be due to hydrophobic interactions between the protein and the surface.

Grazing Angle Infrared Analysis. Grazing angle infrared spectroscopy has been used in this study to assess the conformational state of proteins adsorbed on surfaces modfied with CH_3 groups (hydrophobic) and OH groups (hydrophilic). Infrared spectra of proteins consist mainly of amide band vibrations, Figure 5.

The amide I region ($\sim$1700−1600 cm^{-1}), largely due to a C═O stretching vibration, has been widely used for conformational studies.[9,19−21] Differences in the amide bond orientations within a protein backbone due to varying secondary structures, α-helices, β-sheet, β-turn, and unordered, give rise to different vibrational frequencies, observed as component peaks which

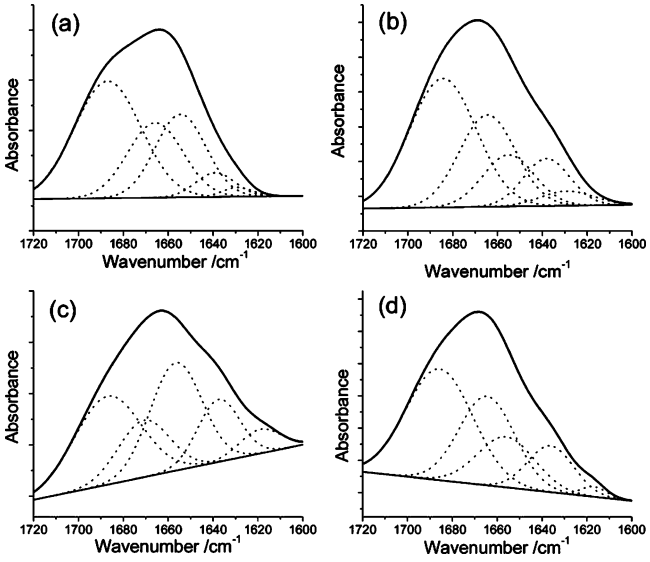

Figure 6. Amide I bands with fitted component peaks: (a) and (b) fibrinogen, (c) and (d) BSA; (a) and (c) OH-terminated surface, (b) and (d) CH_3-terminated surface.

contribute to the characteristic amide I band. The overall shape and maxima of this band are determined by the secondary structure of the protein analyzed.[9,19−21] From previous studies, peaks with maxima at 1685−1663 cm^{-1} have been assigned to β-sheet or β-turn structures, 1655−1650 cm^{-1} to α-helices, 1648−1644 cm^{-1} to random chains, 1639−1635 cm^{-1} to extended chains and 1632−1621 cm^{-1} to extended chains or β-sheets. Bands observed at lower wavenumbers ∼1616 cm^{-1} have been assigned to side chain moieties.[9,20,21]

The amide II region (1500−1600 cm^{-1}) contains information from the in-plane N−H bend and C−N stretch of the amide bond. This band is less sensitive to conformational changes compared to the amide I region, although the amide I/II intensity ratio has been identified as a useful tool to qualitatively assess orientation changes.[9,33] The selection rules for vibrational transitions observed at a grazing angle allow only those dipole moments normal to the surface to absorb. Because the dipole moments of the amide I and II are approximately perpendicular to each other,[34] the ratio of these two bands changes if the adsorbed protein changes conformation. Therefore, time dependent conformational/orientational investigations of surface-bound proteins can be performed. Figure 6 shows example infrared spectra of fibrinogen and albumin attached to CH_3 and OH-terminated surfaces.

Amide I and II maxima and intensity ratios for the proteins adsorbed onto both OH and CH_3 terminated surfaces are presented in Table 2.

No significant changes in either peak position or their relative intensities were observed with time, suggesting that after an initial 1 h adsorption period no further conformational or orientational changes are observed for either protein on either surface.

Results of the amide I band analysis show that protein secondary structure is affected by protein−surface interactions. Both proteins exhibit different conformations on the two underlying surface chemistries, with albumin in particular showing the strongest apparent change with respect to surface chemistry. Figure 6 shows the amide I regions of albumin and fibrinogen attached to CH_3- and OH-terminated surfaces respectively, while conformational data are summarized in Table 3. It is clear from the markedly differing band shapes and the shift of the peak maxima to higher wavenumber on the alkane terminated surface that the proteins are held in differing conformations due to their interaction with the surface.

Table 2. Infrared Data Obtained for Adsorbed Proteins

	incubation time, h	amide I maxima, cm^{-1}	amide II maxima, cm^{-1}	amide I/II intensity ratio
BSA **OH**[a]	1	1662.7 ± 0.3	1541.2 ± 3.2	1.05 ± 0.020
	4.5	1661.2 ± 0.9	1538.2 ± 0.7	1.00 ± 0.005
BSA **CH_3**[a]	1	1667.8 ± 2.5	1545.7 ± 1.9	1.01 ± 0.001
	4.5	1666.5 ± 3.2	1545.4 ± 0.6	1.02 ± 0.001
Fg **OH**	1	1666.3 ± 2.0	1541.2 ± 5.6	1.02 ± 0.016
	4.5	1667.0 ± 0.9	1540.8 ± 2.5	1.03 ± 0.016
Fg **CH_3**	1	1668.2 ± 1.0	1547.3 ± 0.7	1.04 ± 0.002
	4.5	1666.4 ± 1.1	1544.7 ± 1.9	1.05 ± 0.003

[a] **OH** and **CH_3** represent hydrophilic and hydrophobic surfaces, respectively.

Curve fitting analysis of component bands shows that on the alkane surface, proteins lose a large percentage of their α-helical character, shown by a decrease in the percent area of the component band centered at ∼1655 cm^{-1}. An increasing contribution from bands at higher wavenumber indicates a concurrent increase in the β-sheet or random structure component. This behavior was observed to a greater extent for albumin than fibrinogen. The component band centered at 1638 cm^{-1} for fibrinogen was larger on the alkane surface, which can be related to the extended chain conformation of this protein. These findings suggest that both proteins are somewhat denatured on interaction with a hydrophobic surface, losing a large degree of their helical secondary structure. This change in conformation may arise from a stronger interaction with the surface due to hydrophobic interaction, a feature that has been previously reported for both BSA and fibrinogen along with other proteins, studied by SPR[17] and AFM.[18]

The exposure of a hydrophobic protein to water is energetically unfavorable. While the enthalpy of this interaction is small and negative, the Gibbs free energy will increase as a result of the negative entropic contribution ($\Delta G = \Delta H - T\Delta S$). As a protein molecule interacts with a surface, the energy of the system is minimized. Here a large positive change in entropy occurs on the loss of water from the protein and from the surface. When the surface is hydrophobic, adsorption is even more energetically favorable. It follows that interaction of a protein with a hydrophobic surface is expected to be greater than toward a hydrophilic surface, unless the protein in question contains few hydrophobic regions on its surface. Exclusion of water also allows a greater proportion of the protein to interact with the surface, which may help to explain the greater conformational change of albumin on the hydrophobic surface. Upon binding, hydrophobic surface interactions from the outer layer and possibly from the inner core of the protein may occur. The protein will be distorted as the structure deforms to maximize any such surface interactions, Figure 4a. The degree of conformational change will therefore be dependent on the protein−surface interactions and the internal bonding strengths holding the protein in its particular conformation.

(33) Kim, H. S.; Hartgerink, J. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1998**, *120*, 4417−4424.
(34) Miyazawa, T.; Blout, E. R. *J. Am. Chem. Soc.* **1961**, *83*, 712−719.

Table 3. Conformational Analyses of Adsorbed Proteins on OH (hydrophilic) and CH_3 (hydrophobic) Surfaces. Structure Percentage Values Are Given

	BSA **OH**[a]		BSA **CH_3**[a]		fibrinogen **OH**		fibrinogen **CH_3**	
(% structure) incubation time	1 h	4.5 h	1 h	4.5 h	1 h	4.5 h	1 h	4.5 h
β-sheet/random	53 ± 4.7	52 ± 2.5	70 ± 1.7	69 ± 1.2	64 ± 2.8	62 ± 6.9	68 ± 2.4	73 ± 1.8
α-helix	32 ± 1.9	34 ± 0.4	17 ± 0.9	17 ± 0.6	25 ± 2.7	23 ± 3.0	16 ± 2.4	15 ± 1.2
β-turn/sheet	15 ± 1.5	14 ± 1.6	13 ± 1.7	14 ± 0.6	10 ± 3.7	13 ± 2.2	15 ± 2.7	12 ± 0.4

[a] **OH** and **CH_3** represent hydrophilic and hydrophobic surfaces, respectively.

In a similar argument to that above, fibrinogen molecules may adsorb with their axis parallel to the surface, although a minimum energy could be achieved if the fibrinogen molecules stacked to reduce their interaction with water, Figure 4. Some evidence for this type of behavior is provided by the multistage adsorption process as measured by QCM.

Conclusion

In summary, this study illustrates the use of QCM and grazing angle infrared spectroscopy for the study of protein−surface interactions. The combination of techniques is applicable to all biomaterial investigations including studies of cell adhesion where cells interact with the surface via an adsorbed protein layer. In this model study, serum albumin and fibrinogen readily adsorb onto hydrophilic (OH-terminated) and hydrophobic (CH_3-terminated) surfaces. Albumin has a much higher binding affinity toward the hydrophobic compared to the hydrophilic surface, having a lower degree of ordered structure when adsorbed onto the former. In contrast, fibrinogen adsorbs more rapidly to both surfaces compared with albumin and is less deformed, although IR data suggest that adsorption-induced deformation occurs to a greater extent on the hydrophobic surface. Both proteins show rapid initial adsorption (within 1 h), after which time few time-dependent conformational changes are apparent.

At high concentrations (>0.5 mg mL^{-1}) fibrinogen undergoes a multistage adsorption process, unlike albumin which was found to adsorb in a single stage process at the concentrations used. This suggests a rearrangement or orientation change for fibrinogen upon binding, possibly due to protein−protein interactions. Adsorbed fibrinogen molecules may reorient, moving their long axis perpendicular to the surface, a rearrangement driven by increased hydrophobic interaction between adsorbed molecules, Figure 4.

This study highlights the ability to control protein conformation and orientation upon adsorption through surface-protein interactions. By further understanding the interface between biomaterial and biomatter, protein and subsequent cell adhesion may be regulated.

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