Competitive protein adsorption at radio frequency plasma polymer surfaces

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The competitive adsorption of human serum ablumin (HSA), IgG and fibrinogen (Fgn) at radio frequency plasma polymer surfaces was studied with *in situ* ellipsometry and TIRF (total internal reflection fluorescence spectroscopy). While both IgG and Fgn adsorbed preferentially over HSA at hydrophobic surfaces, such as P-HMDSO (monomer: hexamethyldisiloxane), preadsorption of the latter protein dramatically reduced the adsorption of the two former. Furthermore, the preadsorbed HSA is not exchanged by HSA added after preadsorption, i.e., the HSA "blocking" is due to an irreversible adsorption of this protein, presumably involving conformational changes of HSA on adsorption. Different plasma polymer surfaces behave differently regarding competitive protein adsorption. For example, at P-HMDSO, the adsorption from all mixtures investigated is substantial. Furthermore, HSA and IgG dominate the adsorption from a ternary mixture corresponding to 1/100 plasma, although Fgn is still present at the surface. At P-AA (monomer: acrylic acid), on the other hand, the adsorption is lower, except for the IgG/Fgn binary mixture, and Fgn and HSA dominate the adsorption from a ternary mixture corresponding to 1/100 plasma, while IgG is depleted from the surface.

1. Introduction

An important event on contact between a biomaterial and a biological fluid is the adsorption of proteins, which often triggers further biological responses, such as complement activation and blood coagulation. Obviously, the amount of proteins adsorbed is of major importance for these events. However, the interfacial conformation of the adsorbed proteins is also of importance, and it is expected that a protein which encounters a surface will change its conformation sufficiently to cause a biological response. Furthermore, since the protein adsorption occurs from a complex mixture of proteins, the composition of the adsorbed layer will vary strongly between different materials, and hence cause different biological responses. For example, a surface which preferentially adsorbs albumin is expected to be favourable, e.g. for blood contact. In this communication, we report on some results obtained regarding this competitive adsorption from model protein mixtures at plasma polymer surfaces.

2. Experimental procedures

2.1. Materials

Human serum albumin (HSA), globulin free, lyophilized and crystallized, was obtained from Sigma Chemical Co., USA, as was reagent grade purified immunoglobulin (IgG) and fibrinogen (Fraction I; 92% clottable protein). All proteins were used without further purification.

Labelling of the proteins was made with fluorescein isothiocyanate (FITC; Isomer I, Molecular Probes, USA). The labelled proteins were purified from unbound FITC by gel chromatography (PD 10, Pharmacia, Sweden). The effect of the labelling on the interfacial behaviour of the proteins was tested by surface tension measurements using the pendant drop method. No effect on the surface tension was observed for the labelling density used here (below unity calculated as molar ratios).

Chemicals used for the buffer preparation were all of analytical grade, and used without further purification.

2.2. Surfaces

For the ellipsometry measurements, polished silicon slides, thermally oxidized to give an oxide layer of about 30 nm, were used, while for the TIRF experiments, microscope slides from boron silicate (Clay Adams, USA) were used.

The diamino-cyclohexane (P-DACH), acrylic acid (P-AA) and hexamethyldisiloxane (P-HMDSO) surfaces were all prepared by radio frequency glow discharge plasma polymerization. A thorough description of the surface preparations is given in [1].

The methylated silica (Me-Si) surfaces were prepared by covalent attachment of $Cl_2(CH_3)_2Si$ to the silanol groups of the pretreated silicon oxide layer [2].

2.3. Ellipsometry

The ellipsometry measurements were all performed by means of null ellipsometry. The instrument used was an automated Rudolph thin-film ellipsometer, type

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436, controlled by a personal computer. A xenon lamp, filtered to 401.5 nm, was used as light source. A thorough description of the instrument, the methodology used and the theory of four-zone null ellipsometry experiments, as well as of adsorption studies at layered substrate surfaces, is given in [3–5].

In short, the measurements are initiated by an optical analysis of the bare substrate surface, whereafter the protein solution is added, and the (total) adsorbed amount measured as a function of time. All adsorption experiments were performed from 0.01 M phosphate buffer, 0.15 M NaCl, pH 7.2.

2.4. TIRF

TIRF (total internal reflection fluorescence spectroscopy) was used to selectively observe the adsorption of one protein from protein mixtures. The details of the present equipment as well as quantification details, etc. will be discussed in a separate communication [6].

The TIRF experiments were initiated by rinsing the cell with buffer solution. A solution containing 1 µg/ml of sodium fluorescein was then introduced into the cell. By this procedure the instrument could be properly aligned at the same time as an internal standard was introduced into the cell. The cell was then rinsed with buffer until no fluorescence could be detected. After this the protein solution was introduced and the fluorescence intensity detected as a function of time. Again, all adsorption experiments were performed from 0.01 M phosphate buffer, 0.15 M NaCl, pH 7.2.

3. Results and discussion

3.1. Surface characterization

The plasma polymer surfaces prepared have been characterized using several different techniques, and the results from this characterization are summarized in Table I. For details, see [1, 7–9].

As can be seen from Table I, P-AA and P-DACH are both hydrophilic and fairly highly, although oppositely, charged. P-HMDSO, on the other hand, is strongly hydrophobic, although slightly negatively charged.

3.2. Binary mixtures studied with ellipsometry and TIRF

In order to facilitate studies of the mechanisms of competitive protein adsorption at plasma polymer surfaces, we started our investigation with model protein mixtures. For example, the adsorption from binary mixtures of HSA, IgG and fibrinogen was studied at hydrophobic surfaces such as P-HMDSO and Me-Si [10].

The simultaneous adsorption of HSA and either fibrinogen (Fig. 1a) or IgG showed a preferential adsorption of the latter proteins. However, if HSA was allowed to made good contact with the surface, neither fibrinogen (Fig. 1b) nor IgG could displace HSA to any large extent. Furthermore, HSA was not displaced by other HSA molecules after an incubation time of 2 h. (Fig 1b.). These findings indicate that HSA, if given time, will adsorb irreversibly at low energy surface. Most likely, this irreversible adsorption involves surface-induced conformational changes of the protein.

TABLE I Surface properties of freshly prepared plasma polymers

	P-AA	P-DACH	P-HMDSO
Monomer	Acrylic acid	1,2-diamino cyclohexane	hexamethyl disiloxane
Structure of monomer	$H_2C{:}CH{-}C(=O){-}COOH$	cyclohexane ring with two NH_2 groups	$(H_3C)_3Si{-}O{-}Si(CH_3)_3$
Wettability	Hydrophilic < 20° [a]	Hydrophilic < 20° [a]	Hydrophobic 100° [a]
Surface charge [b] Z-potential	negative pH 3-11	positive pH 3-11	negative pH 3-7
pKa and pKb [c]	$pK_a \approx 3$	$pK_b \approx 9,6$ $pK_a \approx 4.6$	$pK_a \approx 7.5$
Area/charge [d] (nm^2)	7.6	7.3	57.2
Refractive index	1.51 [e]	1.51 [e]/1.53 [f]	1.47 [e]
Composition [g]			
C/O/N/Si (exp)	75/25/0/0	87/3/10/0	57/22/0/21
C/O/N/Si (theor.)	60/40/0/0	75/0/25/0	67/11/0/22

[a] Advancing contact angle of sessile water drop [1, 7–8].
[b] As observed from electro-osmosis at a flat plate [9].
[c] As observed from the pH dependence of electroosmotic fluid flow [9].
[d] Calculated as diffuse double layer charge from elektrokinetic measurements [9].
[e] Measured with ellipsometer [1].
[f] Measured in the surface force apparatus [8].
[g] ESCA [1]. Relative atomic composition of the outermost 5–10 nm of the plasma polymer.

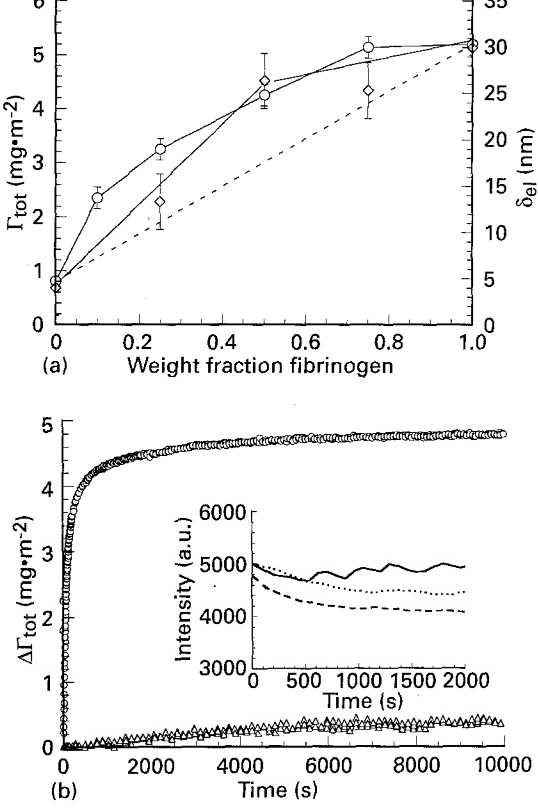

Figure 1 (a) Total adsorbed amount (○) and adsorbed layer thickness (◇) from HSA/Fgn binary mixtures (C_{tot} = 200 ppm) at methylated silica versus the binary mixture composition. (b) Total adsorbed amount difference on adsorption of fibrinogen at methylated silica surfaces. Measurements were made with (△) and without (○) preadsorption of HSA (200 ppm) for 90 min followed by rinsing with buffer for 30 min prior to Fgn addition (100 ppm). Shown also (insert) is TIRF intensity versus time. Measurements were made by adsorbing labelled HSA (200 ppm) at methylated surfaces for 90 min, followed by rinsing with buffer, and subsequent addition of unlabelled HSA (———), IgG (– – – –) or Fgn (·····). The bulk HSA concentration after rinsing was less than 10 ppm.

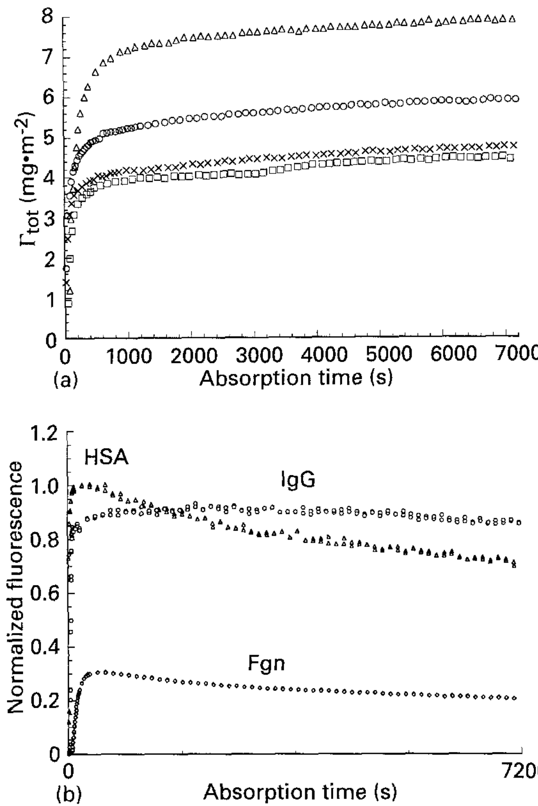

Figure 2 (a) Total adsorbed amount on adsorption at P-HMDSO from binary and ternary mixtures of HSA (A), IgG (I) and Fgn (F) at concentrations corresponding to 1/100 plasma: □ AF; ○ AI; △ IF; × AIF. (b) Normalized fluorescence intensity on adsorption from the ternary mixture at P-HMDSO.

3.3. Ternary mixtures studied with ellipsometry and TIRF

The next step in our studies of the competitive protein adsorption at plasma polymer surfaces was to increase the protein mixture complexity somewhat and to study the simultaneous adsorption from a ternary protein mixture [6, 11]. In both the ellipsometry and TIRF experiments we used a mixture of HSA, IgG and Fgn at concentrations corresponding to 1/100 of those in plasma. In each TIRF experiment, one of the three proteins was labelled with FITC. As substrates, we used P-AA, P-DACH and P-HMDSO.

The TIRF measurements show that IgG and HSA dominate at the P-HMDSO surface, but Fgn was also present on the surface (Fig. 2b). The surface concentrations of the three proteins remained almost constant during the experiment (2 h) indicating that there is little exchange between the different proteins on this time scale. It is clear that HSA and/or IgG at this surface and at these concentrations succed in blocking the adsorption of Fgn to a large extent. This is further indicated by the finiding of the total amount of protein adsorbed at this surface from the ternary mixture being only 4.4 mg/m^2, which is less than the adsorp-

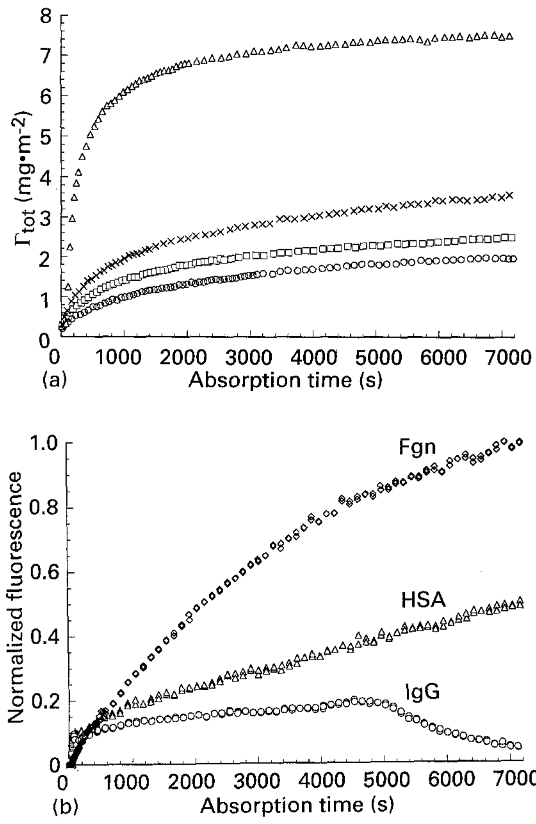

Figure 3 (a) Total adsorbed amount on adsorption at P-AA from binary and ternary mixtures of HSA (A), IgG (I) and Fgn (F) at concentrations corresponding to 1/100 plasma: □ AF; ○ AI; △ IF; × AIF. (b) Normalized fluorescence intensity on adsorption from the ternary mixture at P-AA.

tion of either Fgn or IgG from the single protein solutions (Fig. 2a) [11].

At P-AA the adsorption from single protein solutions is less than at P-HMDSO and P-DACH, the difference being largest for IgG and Fgn [11]. Furthermore, for both P-HMDSO and P-DACH, the rate of adsorption is much faster than that for P-AA. It was found that the mixture of IgG and Fgn results in an adsorbed amount much higher than that of the HSA/IgG and HSA/Fgn mixtures (Fig. 3a), as well as that of the ternary protein mixture. The TIRF experiments show that Fgn and HSA dominate the adsorption at this surface with Fgn being the protein adsorbing most extensively (Fig. 3b). Both the HSA and Fgn adsorbed amounts increase during the whole experiment (2 h) while IgG is completely depleted from the surface.

P-DACH shows similar adsorption kinetics as P-HMDSO (results not shown), but the mixtures HSA/Fgn, IgG/Fgn and HSA/IgG/Fgn give extremely high adsorbed amounts, approximate 17 mg/m^2 [11]. For these mixtures, the protein adsorption occurs in multilayers. The TIRF experiments show that Fgn dominates at this surface, but that both HSA and IgG are present at the surface as well.

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