Antibody Binding to Antigen-Coated Substrates Studied with Surface Plasmon Oscillations

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We have used surface plasmon oscillations to monitor the time evolution of the adsorption of antibodies from aqueous solution onto an antigen-coated surface. The asymptotic adsorbance values (Γ_f) do not reach a maximum until the concentration of antibody in solution (C) is greater than 1 mg/mL. We interpret the shape of the Γ_f vs C plot as an evolution from side-on adsorption to end-on adsorption. Our data show a characteristic time, t_c, which is proportional to C^{-2}, indicating diffusion-limited adsorption. The value of t_c is significantly greater than predicted from a diffusion-limited model. We attribute this result to a requirement that the antibodies be correctly oriented if they are to be adsorbed. A careful study of our kinetics data shows that for high concentrations we must apply theories of random sequential adsorption in addition, because the adsorbed molecules may exclude more surface area than they physically occupy.

Introduction

The adsorption of proteins at the solid/liquid interface is of fundamental and practical interest,[1-8] having relevance to such areas as food processing, contact lenses and medical implants, protein purification and processing, and immunoassays. The interaction of a protein with a surface is strong, particularly so if the surface and the protein are a layer of preadsorbed antigen and its antibody, respectively. Many investigations[9-20] of protein adsorption involve placing the surface in contact with an aqueous solution which contains small quantities of the protein one wishes to study. Despite the high binding energies, it is generally the case that the amount of material adsorbed from solution increases only gradually as the quantity of protein in the solution increases; the isotherm is a smoothly increasing function of the protein concentration in the solution. Numerous models have been proposed to account for the observed behavior; most assume that the system reaches an equilibrium state which depends on changes in the adsorbed molecules ("denaturing"). The validity of these models can be tested by observing the time dependence of the adsorption process, but most experimental work has only measured the final values of the adsorbance.[1,3]

We have used surface plasmon oscillations (SPO's) to examine the time evolution of antibody adsorption onto an antigen-coated surface over a wide range of antibody solution concentrations. Our technique permits us to measure the surface density of adsorbed antibody for times ranging from several tens of seconds to 1 day or more. No labeling of the relevant molecules was required, and the measurements were made *in situ*. The antigen was bovine serum albumin (BSA) and the antibody was rabbit anti-BSA (RABSA).

Our data are well-characterized by a stretched-exponential time dependence for the adsorbance as a function of time. This function is similar to the stretched error function used by Mayo[21] in an earlier study of RABSA adsorption on BSA. We fit our data to the stretched exponential form because it permits direct comparison between our data and simple models of adsorption kinetics. We find an inverse-square relationship between antibody concentration and characteristic adsorption time.

Experimental Technique

The SPO technique[22] involves the interaction of an evanescent photon field with the boundary condition found at the interface between a thin metal film and a dielectric material. The SPO device we have used[21] is configured in the manner described by Kretschmann.[23] A thin layer of gold (400 Å) is deposited on the plane face of a hemicylindrical optical prism (type SF-10, n = 1.72, J.R. Cumberland). A plane-polarized light beam from a helium-neon laser (Uniphase 1105P, λ = 6328 Å) is incident normal to the curved face of the prism (see Figure 1). The light beam penetrates the prism and reflects from the metal/prism interface, creating an evanescent field at the metal/dielectric interface. This evanescent field can couple with the surface plasmon waves propagating on the metal surface. When the wave vector of the incident light matches with the wave vector

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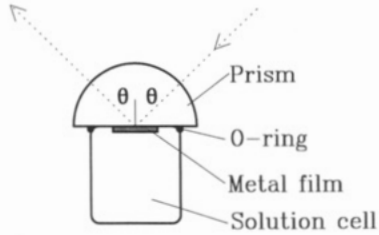

Figure 1. Basic geometry of sample cell showing the Kretschmann SPO configuration. Plane-polarized light (- -) is incident on the metal-coated face of the prism at angle θ. The intensity of the reflected signal is read by the photodetector (not shown).

of the surface plasmons, a resonance occurs and the reflected beam is strongly attenuated. In practice, this can be achieved by changing the incident angle of the light until a minimum occurs in the reflected signal (at θ_{SPO}). We accomplish this by mounting the prism and the laser on a rotating table (a Siemens type F, $\theta - 2\theta$ goniometer). The reflected signal is read by a photodiode (United Detector Technology PN-6DP); the photodiode's output voltage is amplified (Texas Instruments μA747) and digitized (Hewlett-Packard 3478A). A computer (Epson Equity III+) controls a stepper motor which rotates the goniometer in 0.01° increments; it then records the angle and the voltage from the multimeter. This is repeated until the signals from a specified range of angles has been recorded. Our device allows us to determine θ_{SPO} at intervals as short as 36 s. Higher temporal resolution is available.[24]

The value of θ_{SPO} depends on the refractive index of the medium in contact with the metal surface. The SP field is very sensitive to changes in the interfacial region, as it only penetrates ≈1800 Å into the medium. If a thin film forms on the surface, there will be a shift in θ_{SPO} proportional to the mass adsorbed per unit area, Γ.[25]

A fresh gold film was used for each adsorption process studied. Films were made in a vacuum evaporator (Norton Vacuum 3115). The evaporator bell jar was flushed twice with nitrogen from the ventline of a liquid nitrogen dewar; in this way, the metals were deposited in a nitrogen residual atmosphere. To enhance the gold film's adhesion to the prism, roughly 75 Å of copper was deposited on the prism *via* a plasma etch at 5×10^{-2} Torr and 3600 V. The quantity of copper deposited was determined by a quartz crystal thickness monitor (Sloan Omni III). When the pressure was 3−7 μTorr, roughly 400 Å of gold was deposited at a rate of 25−40 Å/s, as determined by the thickness monitor. The prism was allowed to cool for at least 2 h following evaporation. Within 20 min of removal from the bell jar, a small glass cell (see Figure 1) was clamped to the metal-coated prism face and used to hold a solution; a Teflon o-ring made the seal leak-tight. The prism/cell assembly was enclosed in a temperature-controlled oven, where the temperature was maintained at 30 ± 0.05 °C throughout the course of the experiments. The BSA antigen (Sigma A-7030) and the RABSA antibody (Sigma B-7276) were dissolved in phosphate-buffered saline (PBS; Sigma P-4417, pH = 7.4). The BSA solution concentration was 2 mg/mL; the RABSA solutions had concentrations ranging from 8800 to 340 μg/mL (later diluted for our measurements). As soon as a cell was clamped to a prism, it was filled with 3−5 mL of BSA solution (using a syringe) and monitored by the SPO device for 12−16 h. The BSA typically forms a film within 5 min. We performed an experiment in which PBS only was injected into the cell, θ_{SPO} was determined, and then the BSA/PBS solution was added while we monitored $\Delta\theta_{SPO}$. We found that $\Delta\theta_{SPO}$ reached an asymptotic value of approximately 0.4° within 5 min. Many of the samples showed a slow decrease in θ_{SPO} ($\Delta\theta_{SPO} \leq -0.12°$ during the first 12 h, after which θ_{SPO} stabilized). These shifts may be due to partial detachment of the metal film from the glass prism. Another possible explanation is that the BSA may have the effect of displacing contaminants on the metal surface. Since these changes are occurring only very slowly by the time we add the antibody, they are not a significant factor in our experiment.

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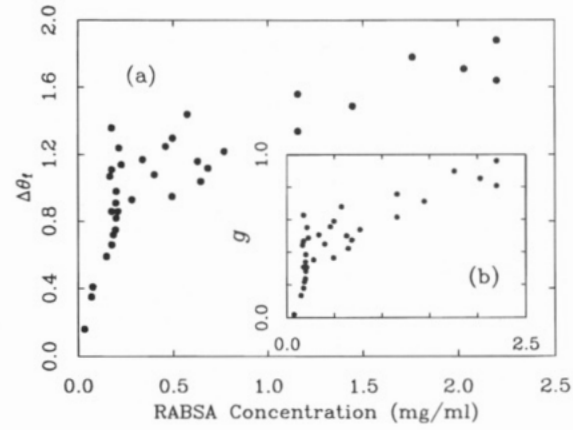

Figure 2. (a) Asymptotic values of the surface plasmon resonance shift ($\Delta\theta_f$) for RABSA adsorbing onto a BSA-coated gold surface from PBS (T = 30 °C, pH = 7.4). Inset b shows fraction g of end-on adsorbed RABSA as calculated using eq 1. Negative values of g for $C \leq 70$ μg/mL are not shown.

Prior to adding the antibody, the BSA solution in the cell was removed and replaced with PBS. This was repeated twice to ensure that there was only a residual quantity of BSA molecules left in solution. This change θ_{SPO} very slightly (±0.02°). Once a baseline value for θ_{SPO} was obtained, a small quantity (≤1 mL) of RABSA/PBS concentrate ($340 \leq C_{conc} \leq 8800$ μg/mL) was added to the PBS filled cell. The resulting solution ($33 \leq C \leq 2200$ μg/mL) was agitated briefly to ensure an even distribution of RABSA, and the change in the resonance, $\Delta\theta_{SPO}$, was monitored continuously. Data were taken for 24 h, and the value of $\Delta\theta_{SPO}$ at t = 24 h was taken as the final value ($\Delta\theta_f$).

We considered that our results might contain systematic errors if the RABSA solutions were not sufficiently agitated upon addition to the cell. If the RABSA molecules were not evenly distributed throughout the cell volume, we would obtain skewed results owing to excessively long or short distances required for them to reach the BSA surface. To this end, we conducted an experiment in which we removed most (all except ≈ 0.2 mL) of the PBS from the cell and filled it completely with RABSA solution ($C_{conc} \approx C$ = 497 μg/mL). The resulting data were consistent with results obtained after injecting small quantities of a highly concentrated solution into the PBS-filled cell and agitating it to obtain comparable values of C. Thus we conclude that our solutions were sufficiently agitated.

For several data sets, we attempted to reverse the adsorption by replacing the RABSA solution with clean PBS. When this was done, no change in θ_{SPO} was observed during 20 h of observation. We conclude that for our experimental conditions the adsorption is essentially irreversible.

Detailed Results

Asymptotic Behavior. Our isotherm ($\Delta\theta_f$ *vs* RABSA solution concentration, C) for a temperature of 30 °C is shown in Figure 2a. RABSA molecules are primarily IgG, which have dimensions of 235 × 44 × 44 Å and a molecular weight of 160 000 (BSA molecules have a molecular weight of 73 000). Thus a complete monolayer of RABSA is expected to have a maximum adsorbance of $\Gamma_* = 1.37$ μg/cm^2.[12] This is the density for IgG molecules adsorbing end-on to the surface (as opposed to side-on adsorption). The corresponding shift in θ_{SPO} is expected to be $\Delta\theta_* = 1.94°$ if we assume $\partial n/\partial C \approx 0.19$ cm^3/g,[12] where n is RABSA's index of refraction and $\partial n/\partial C$ is the rate of change of the refractive index with respect to a change of the protein concentration in PBS. Our maximum shift (1.88°) is close to the calculated value. Similar quantities for RABSA adsorption on BSA were found by Nygren and Stenberg using ellipsometry and an enzyme-linked immunosorbent assay.[15] Our work covers higher concentrations and provides greater detail of the time dependence.

Several studies of protein adsorption are interpreted

in terms of the Langmuir model.[1,9,26] However, our adsorbance values are asymptotic ($t \rightarrow \infty$) values, which must be distinguished from so-called "equilibrium" values. The Langmuir model assumes a reversible process in which the final adsorbance values reflect a dynamic equilibrium in which the rates of adsorption and desorption are equal. Our data indicate that adsorption is irreversible; a different mechanism is responsible for the shape of the isotherm.

We note the model described by Morrissey.[14] At low concentrations, there is a significant interval between arrival times for RABSA molecules. During this interval, a newly adsorbed molecule, which initially has acquired only a one-site "foothold" on the BSA, has adequate time to bind more strongly using other domains on its surface. It therefore will likely bind in a so-called "side-on" mode, in which the protein has unfolded into a "pancake" on the surface. At very high concentrations the adsorbing molecules only have time to bind with a single site; we expect them to be weakly attached to the surface, but packed tightly in an "end-on" configuration. Figure 2b (inset) shows values for a parameter, g, which represents the fraction of molecules adsorbed in the end-on mode as concentration changes:

$$\Delta\theta_f = g\Delta\theta_{end} + (1 - g)\Delta\theta_{side} \quad (1)$$

For our fits, we used $\Delta\theta_{end} = 1.94°$ and $\Delta\theta_{side} = 0.38°$. These values correspond to $\Gamma_{end} = 13.7 \times 10^{-7}$ g/cm^2 and $\Gamma_{side} = 2.7 \times 10^{-7}$ g/cm^2.[12] At the lowest concentrations we examined ($C \leq 70$ μg/mL), g was found to be less than zero. This indicates that for very low solution concentrations there is some competition for adsorption sites between the RABSA molecules and the ions in the PBS, and thus eq 1 is only applicable for $C \gg 70$ μg/mL.

Kinetics. When the binding energy is very strong, every molecule that makes contact with the surface is adsorbed. Then the adsorption rate is limited by the rate at which molecules can diffuse from the bulk region to the interface. Because the solution adjacent to the interface becomes depleted, the rate of adsorption is reduced because additional molecules must arrive from deeper within the bulk. The adsorption rate is described by

$$\partial\Gamma/\partial t = C[D/(\pi t)]^{1/2}\Phi \quad (2)$$

where D is the diffusion coefficient (typically $2-6 \times 10^{-7}$ cm^2/s for immunoglobulins)[1] and Φ is the fraction of surface available for adsorption. The dependence of Φ on surface coverage is crucial to theories of random sequential adsorption (see below). Φ may have a complicated dependence on Γ, but for now we may approximate it as $\Phi(\Gamma) \approx 1 - \Gamma/\Gamma_f$. Then eq 2 can be integrated to give the adsorbance as a function of time:[15,27,28]

$$\Gamma(t) \approx \Gamma_f[1 - \exp(-(t/\tau)^{1/2})] \quad (3)$$

where $\tau = \tau_D \equiv \pi\Gamma_f^2/4DC^2$.[1,3,27] Here we use Γ_f rather than Γ_* to allow for different configurations of adsorbed molecules at different solution concentrations, as suggested by Morrissey. In the case of molecules which do not adsorb in concentration-dependent configurations one would use Γ_*, but in that case one would also expect the same final adsorbance values at virtually all concentrations greater than zero. Our isotherm does not exhibit this feature, and therefore it is more appropriate to use τ as defined above.

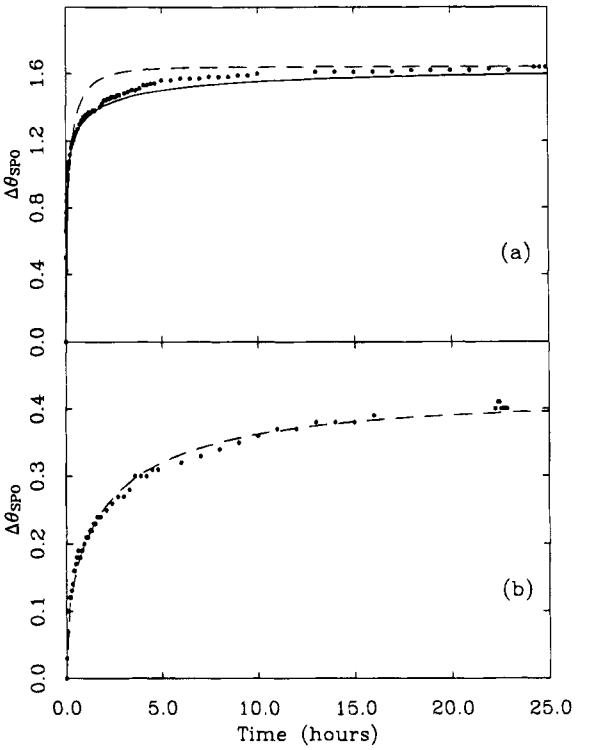

Figure 3. Measured values of $\Delta\theta_{SPO}$ for (a) 2200 and (b) 76.1 μg/mL RABSA/PBS adsorbing on BSA. The solid line in 3a represents the fit to eq 4 using an exponent $\alpha = 1/4$; this yields $\chi^2 = 9.8 \times 10^{-4}$. Dashed lines (- -) in a and b are fits for $\alpha = 1/2$; χ^2 values are 0.012 for a and 8.1×10^{-5} for b.

Our data for high and low antibody concentrations are shown in Figure 3, parts a and b, respectively. Also shown are fits to a stretched exponential function:

$$\Delta\theta_{SPO}(t) = \Delta\theta_f[1 - \exp(-kt^\alpha)] \quad (4)$$

where k is found by a least-squares fit using $\alpha = 1/2$ and $1/4$. The aptness of a fit is measured by the parameter χ^2, which is defined by

$$\chi^2 \equiv \frac{1}{N}\sum_{i=1}^{N}[y_i(\text{fit}) - y_i(\text{data})]^2 \quad (5)$$

for N points with values y_i(data). For the data plotted in Figure 3a ($C = 2200$ μg/mL), the best fit for $\alpha = 1/2$ yields $\chi^2 = 0.012$; however, if we let $\alpha = 1/4$, χ^2 can become as small as 9.8×10^{-4}. For the data shown in Figure 3b ($C = 76.1$ μg/mL), we have $\chi^2 = 8.1 \times 10^{-5}$ for $\alpha = 1/2$. Since the uncertainty in our measurement of $\Delta\theta_{SPO}$ is roughly $\pm 0.015°$, these last two fits provide a satisfactory description of the data.

By allowing α to be a parameter in our fitting, we have determined the optimal values of α for all our data sets; the results are shown in Figure 4. The points indicate the value which minimizes χ^2, with the error bars given by $\chi^2 \leq 1.5\chi_{min}^2$. The largest value of χ_{min}^2 was 2.7×10^{-3}, but for most of our data sets χ_{min}^2 was less than 5×10^{-4}. Recall that for diffusion-controlled kinetics we should find $\alpha = 1/2$. This appears to hold for RABSA concentrations less than 250 μg/mL, but for high concentrations we find $\alpha \rightarrow 1/4$.

We considered the possibility that the change in α may be an artifact of our method. At least 10 s pass before the first data point is taken after addition of RABSA, with

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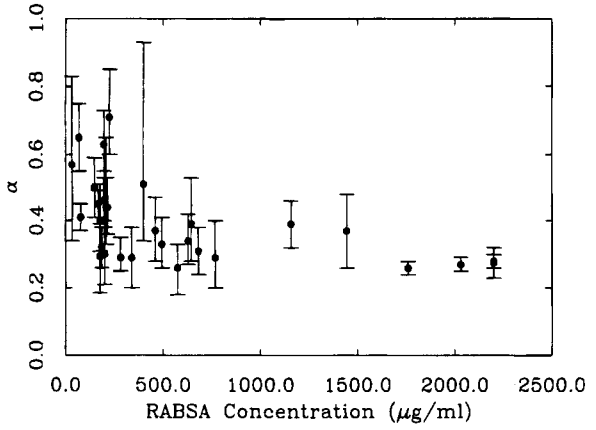

Figure 4. Best values of time exponent α *vs* RABSA concentration: (●) value which yields χ_{min}^2; error bars represent the range within which $\chi^2 \leq 1.5\chi_{min}^2$.

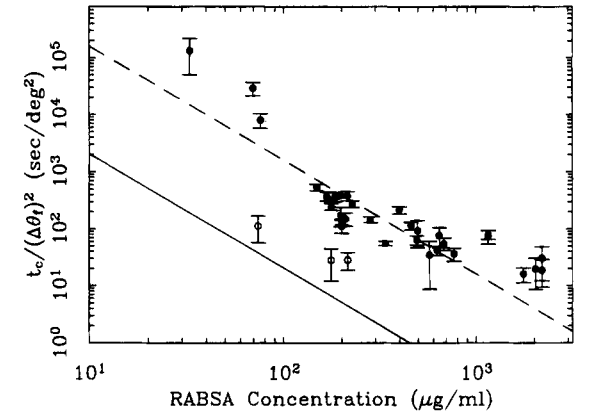

Figure 5. Normalized adsorption time $t_c/(\Delta\theta_f)^2$ *vs* antibody concentration: (●) RABSA on BSA, (○) RABSA on gold. The solid line indicates values predicted by a diffusion-limited model from the right-hand side of eq 7. The dashed line (- -) is a fit to the data assuming $t_c \sim C^{-2}$.

intervals of at least 30 s for subsequent measurements. For low concentrations, these times are not important because θ_{SPO} changes very slowly, and many measurements can be taken while the surface coverage is very low. For high concentrations, however, the coverage has already reached ≈30% of its final value by the time we make our initial measurement. Thus our fits for low concentrations will be weighted toward low-coverage behavior, while for data taken at high concentrations the fits will be weighted in favor of intermediate- to high-coverage behavior. To test for sensitivity to this, we tried fits to low concentration data which neglected measurements taken before $\Delta\theta_{SPO}$ had reached 0.5°. The resulting values of α (not shown) were not significantly altered with the exception of the single data set for $C = 400$ μg/mL, for which we found $\alpha \approx 0.34$ instead of 0.51.

Figure 5 displays the normalized values for a characteristic time, t_c, which we have defined by

$$\Delta\theta_{SPO}(t_c) = \Delta\theta_f/e \qquad (6)$$

and t_c for each data set has been normalized by the value of $(\Delta\theta_f)^2$ which was measured for that data set. Normalizing in this way permits comparison between t_c values which are distributed across a wide range of final adsorbance values (see eq 7, below). The solid circles represent observed normalized t_c values for RABSA adsorbing to a BSA-coated surface; the three hollow circles are the values we observed for RABSA adsorbing onto gold without the intermediate layer of BSA.

By normalizing with $(\Delta\theta_f)^2$ instead of $(\Delta\theta_*)^2$, we allow for a diffusion-limited model which includes Morrissey's hypothesis. In this case, we have final adsorbance values which are concentration-dependent due to unfolding, and we can predict values for t_c:

$$t_c/(\Delta\theta_f)^2 \approx 0.165\Gamma_*^2/D(C\Delta\theta_*)^2 \qquad (7)$$

These values are indicated in Figure 5 by the solid line. It is evident that the adsorption of RABSA on BSA proceeds much more slowly than the calculated diffusion limit. For RABSA adsorbing onto gold, however, the adsorption time is as appropriate for side-on adsorption. The characteristic times and the values of $\Delta\theta_f$ (0.48° and 0.5°) for these data sets indicate that, at the concentrations examined (73.9 and 176 μg/mL), RABSA adsorbs to gold mainly in the side-on configuration.

For RABSA adsorbing to BSA, we may attribute the relative slowness to an orientational requirement for adsorption.[3] A BSA-coated surface is not homogeneous, and a RABSA molecule is neither isotropic in shape nor uniform in chemical structure. Thus it is reasonable to expect that a RABSA molecule arriving at the surface cannot bind to a BSA molecule unless their relative orientations meet specific criteria.

We can obtain an adequate fit to the data of Figure 5 when we multiply the predicted values (from eq 7) by a factor β^2. Thus we may estimate an effective solid angle Ω_{eff} of order $4\pi/\beta$ with $\beta = 8.7 \pm 0.9$. This suggests that a RABSA molecule arriving at a site not already occupied by another RABSA molecule has a probability, equal to $1/\beta$, of binding with the BSA-coated surface. A gold film, by contrast, presents a uniform surface to the RABSA; in this case, $\Omega_{eff} \rightarrow 4\pi$, and the adsorption can proceed much more quickly.

If the RABSA molecules which fail to adsorb upon reaching the interface remain in the region adjacent to the surface, they may be able to reorient themselves and adsorb at a later time. However, the time required to do so may be much greater than the time required for further diffusion of molecules from the bulk. The effective solution concentration will be lower than the actual concentration because most of the RABSA molecules in the solution are directed the wrong way when they reach the surface. If this is the case, then the diffusion-limited model is still applicable to our system.

Discussion

The extreme slowness of the adsorption is only partly explained by the addition of an orientational degree of freedom. This requirement helps to account for a larger value of τ in eq 3, but it does not explain the deviation of the scaling exponent α from $^1/_2$.

The shift in the exponent from $^1/_2$ to $\approx^1/_4$ is most likely due to the shape of the adsorbed RABSA molecules. The appropriate model may be random sequential adsorption (RSA).[29,30] In this model, particles are placed at random on a lattice. Once placed, they are unable to desorb or diffuse along the surface. The adsorption proceeds until a shape-dependent "jamming limit" is reached.[31] This is a reasonable expectation given the strong binding of antibodies to antigens. The starting point for RSA is a generalized Langmuir equation

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$$\partial\theta/\partial t \sim \int d\Omega\, f(\Omega)\Phi[\theta(t),\Omega] \quad (8)$$

where $f(\Omega)$ is the probability distribution for incoming molecules with orientation Ω, and $\Phi[\theta(t),\Omega]$ is the fraction of surface still available for adsorption. In the derivation of eqs 3 and 4, $\Phi[\theta(t),\Omega]$ is approximated by $1 - \Delta\theta_{SPO}/\Delta\theta_f$. For an RSA system, the remaining surface may exhibit a more complicated dependence on surface coverage because adsorbed molecules may exclude more surface than they actually occupy. This effect applies to protein adsorption if there is a mismatch between the antigen site spacing and the antibody molecular size.[3] An additional factor may be that protein molecules may unfold or otherwise reconfigure themselves once they have adsorbed; this has been discussed by Elaissari and Pefferkorn.[32]

Several computational and analytical studies have been made for RSA systems. Poland examined the kinetics of linear chains adsorbing onto a square lattice.[33] He concluded that the adsorption process is slower than exponential because the arriving molecules must not only find the necessary number of binding sites but they must also find these sites in a shape which matches the chain configuration.

Schaaf and Talbot calculated the behavior of $\Phi[\theta(t)]$ for hard disks at intermediate coverage.[30] They found nonlinear terms in Φ which become significant as the coverage approaches its final value. In our experiment, we have assumed that the antibodies adsorb end-on at high concentration. Therefore they present a disk shape to the surface and to additional molecules arriving from the solution. The expression derived by Schaaf and Talbot for surface coverage, $\gamma = \Delta\theta/\Delta\theta_*$, is

$$\Phi_{RSA} \sim 1 - a\gamma + b\gamma^2 + c\gamma^3 + O(\gamma^4) \quad (9)$$

The above equation indicates the probability that an arriving molecule will find an available site. If antibodies were arriving at a constant rate, we could simply integrate to find $\gamma(t)$. We calculated the result using the coefficients provided by Schaaf and Talbot;[30] a fit to eq 4 yields $\alpha \approx 0.6$ for intermediate surface coverage. This value of α may be reduced if higher order terms in the expansion of γ are included. If we assume that diffusion still plays a role, we must divide the right-hand side of eq 9 by $t^{1/2}$. Then we can substitute the following "diffusion-limited random sequential adsorption" (DLRSA) equation for eq 2:

$$\partial\Gamma/\partial t = [1 - a\gamma + b\gamma^2 + c\gamma^3]C(D/\pi t)^{1/2} \quad (10)$$

Equation 10, when integrated numerically using the values of a, b, and c provided by Schaaf and Talbot, produces a function which can fit eq 4 with $\chi^2 = 3 \times 10^{-4}$ if $\alpha \approx 0.3$:

$$\Delta\theta_{SPO}(t) \approx \Delta\theta_f[1 - \exp(-kt^{0.3})] \quad (11)$$

Using eqs 10 and 11, our experiment should yield the same exponent, α, for all solution concentrations if the available surface function, Φ, retains the same coefficients a, b, and c used above. Figure 4 indicates that α changes with concentration; at high concentrations α approaches 0.28, in keeping with the results of Schaaf and Talbot[30] for adsorption of disks (see eq 11). For lower concentrations α is higher; the reason for this is unclear. If the molecules in this regime are adsorbed as ellipses rather than as disks, then we should see α change; the work of Viot *et al.*[31] indicates that α should stay the same or decrease. If, therefore, the molecules are adsorbed in more elongated conformations at low solution concentrations (which is consistent with the isotherm we measured), then the results of Viot *et al.* are not valid for our experiment in this concentration limit.

A more refined model of RSA should taken into account the rate of change of the adsorbed configurations, *i.e.*, one in which the area per molecule increases from the moment the molecule adsorbs until its growth is brought to a halt by an adjacent molecule. In this model, adsorbed proteins behave as fluid molecules which can spread across the surface. This is qualitatively different from the picture used by Gölander and Kiss,[12] in which adsorbed proteins are treated as rigid spheroids whose configurations are determined by the orientation with which they adsorb.

Such a model has been examined by Elaissari and Pefferkorn,[32] but they assumed a constant rate of new molecules arriving at the interface, and their published work does not discuss the kinetics of such a system. A simulation which assumes a diffusion-limited arrival rate at the interface (where the rate decreases with time), and which illustrates the resulting kinetics, would be useful in accounting for our experimental findings. This refined model might enable us to determine the rate of spreading of adsorbed molecules; changes in the rate of spreading might appear as changes in α (Figure 4) and t_c (Figure 5).

Conclusions

By examining the isotherm and the kinetics of a protein adsorption process over a wide range of solution concentrations, we have shown the usefulness of the random sequential adsorption (RSA) concept when coupled with Morrissey's hypothesis.[14] The approach to the asymptotic absorbance value for a given concentration is consistent with diffusion-limited RSA. By modifying the RSA theory of Schaaf and Talbot[30] we can account for the low value of the scaling exponent (α) at high solution concentrations. For lower concentrations our data contradict the prediction of Viot *et al.*;[31] a more dynamical RSA model, similar to the one developed by Elaissari and Pefferkorn,[32] may help explain our results in this region. Such a theory would enable us to use our data to determine whether an adsorbed protein should be treated as a rigid spheroid or as a flexible polymer chain.

We estimate the probability that a molecule arriving at the surface will be adsorbed by measuring the characteristic adsorption time, t_c. We attribute the reduced value of this probability to the existence of small binding domains on the surface of the antibody. Both t_c and α are consistent with diffusion-limited adsorption (*i.e.*, $t_c \sim C^{-2}$ and $\alpha \leq {}^1/_2$).

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