Protein Adhesion Force Dynamics and Single Adhesion Events

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ABSTRACT Using the manipulation force microscope, a novel atomic force microscope, the adhesion forces of bovine serum albumin, myoglobin, ferritin, and lysozyme proteins to glass and polystyrene substrates were characterized by following the force necessary to displace an adsorbed protein-covered microsphere over several orders of magnitude in time. This force was consistent with a power law with exponent $a = 0.37 \pm 0.03$ on polystyrene, indicating that there is no typical time scale for adhesion on this substrate. On glass, the rate of adhesion depended strongly on protein charge. Forces corresponding to single protein adhesion events were identified. The typical rupture force of a single lysozyme, ferritin, bovine serum albumin, and myoglobin protein adhering to glass was estimated to be 90 ± 10 pN, 115 ± 13 pN, 277 ± 44 pN, and 277 ± 44 pN, respectively, using a model of the experimental system. These forces, as well as the force amplitudes on hydrophobic polystyrene, correlate with protein stiffness.

INTRODUCTION

Proteins take part in specific interactions with other proteins, as in the immune system and in cell adhesion, but may also form strong nonspecific interactions with a surface. This property is widely used in the biological sciences, where several experimental techniques depend on protein immobilization on a surface. Protein adhesion is also of theoretical interest, because its strength depends strongly on protein structure.

Interactions between proteins have been studied in great detail in biology but investigations of the mechanical properties of interacting proteins were only recently made possible by force microscopy techniques such as the surface force apparatus (Israelachvili and Adams, 1976; Claesson et al., 1995) and the atomic force microscope (AFM) (Binnig et al., 1986).

The AFM has been particularly useful in studying interactions between biological molecules. The force between biotin and streptavidin (Lee et al., 1994; Florin et al., 1994) and between immunoglobulin G and its antigen (Dammer et al., 1996; Allen et al., 1997; Browning-Kelly et al., 1997; Perrin et al., 1997), was measured at single-molecule resolution. The AFM has also been used to recognize areas with adsorbed protein on polystyrene (Chen et al., 1997), and to investigate the mechanical properties of adsorbed titin proteins (Rief et al., 1997).

The manipulation force microscope was recently introduced (Sagvolden et al., 1998, 1999a). This novel AFM measures the force necessary to displace an object adhering to a substrate. It was first used for studying the characteristic adhesion forces of proteins to glass and to hydrophilic and hydrophobic polystyrene (Sagvolden et al., 1998). Recently, it has been used to study the adhesion of living cells to substrates (Sagvolden et al., 1999b).

This force microscope is particularly well suited to studying the dynamic evolution of adhesion forces over a wide range of time scales. In the conventional AFM technique, the specimen is linked between the cantilever force transducer and the substrate. This geometry is sensitive to mechanical noise and thermal drift, which complicates the study of slow dynamics. In the manipulation force microscope, the specimens are distributed on the substrate and in contact with the cantilever only during force measurement. Proteins are carried on microspheres that are allowed to adhere on the substrate and the adhesion force of each microsphere may be measured. This allows for the study of adhesion force dynamics on time scales ranging from seconds to hours, which is difficult using conventional techniques.

In this study the manipulation force microscope was used to measure the adhesion forces of proteins to glass and polystyrene as a function of the contact time between the substrate and the proteins. On glass, where adhesion probability is low, forces due to a single protein adhesion event were identified.

MATERIALS AND METHODS

Experimental

Microsphere preparation and instrumentation followed techniques described elsewhere (Sagvolden et al., 1998, 1999a). In brief, silica microspheres 4 μm in diameter (Bangs Laboratories, Fishers, IN) were functionalized by exposure to a 5% (γ-aminopropyl)triethoxysilane (Fluka, Buchs, Switzerland) solution for 2 hours, then a 2.5% glutaraldehyde (Fluka) solution for 2 hours. Bovine serum albumin (BSA, Sigma, St. Louis, MO) horse spleen ferritin (FER, Sigma) hen egg white lysozyme (LYS, Sigma), or horse skeletal myoglobin (MYO, Sigma) proteins at a concentration of 1 mg/ml in TBS (33 mM TRIS; 100 mM NaCl), pH 8.0, were then linked to the microspheres.

Hydrophobic polystyrene substrates were cut from sterile tissue culture dishes (Nunc, Roskilde, Denmark). Clean glass substrates (Menzel, Braunschweig, Germany) were sterilized by heating to 300°C. Liquid cells were made by gluing (LC 3068, Castall, East Weymouth, MA) an 11 × 3-mm

Received for publication 17 November 1998 and in final form 5 April 1999.
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0006-3495/99/07/526/07 $2.00

Viton O-ring (Busak & Shamban, Ft. Wayne, IN) to the glass substrates, while surface forces held the O-ring in place on polystyrene.

At start of the experiment a small amount of microspheres was injected into the TBS-filled liquid cell to give 1 sphere per 100 μm^2 of surface area. The spheres were subsequently displaced using an AFM cantilever (Nanoprobes, Digital Instruments, Santa Barbara, CA) glued (LC3068, Castall) at an angle of 30° to the vertical (Fig. 1 *A*) to a top glass slide closing the liquid cell. Cantilever deflection was detected using a fiber optic laser (SK 9660, Schäfter & Kirchhoff, Hamburg, Germany), a beam splitter cube (03BSD044, Melles Griot, Rochester, NY), and a split diode (SD 113-24-21-021, Advanced Photonix, Camarillo, CA) in an autocollimator arrangement. The diode signal was amplified, measured with a multimeter (2001, Keithley, Cleveland, OH) at a rate of 1 kHz, and logged by a PC. The sample was translated continuously using a micromanipulator (MO-302, Narishige, Tokyo) and a geared motor (Multur, Halstrup, Kirchzarten, Germany) at velocities from 0.025 to 8.3 μm/s. The optical detection system was characterized using a gold wire glued to the bottom of a liquid cell. The cantilever force constant was found to be 0.40 N/m using a gold wire, 13 μm in diameter, as compliance standard.

Adhesion time

Adhesion forces were measured as a function of the time the proteins had been in contact with the substrate. This time was controlled by three different methods. To observe very slow dynamics, adhesion was measured as a function of the time passed after the microspheres were seeded on the substrate. Intermediate adhesion times from 5 to 2560 s were obtained by first displacing the microsphere to detach it from the substrate, then letting the proteins interact with the substrate for an adsorption time Δt, and finally displacing it at a rate of 0.83 μm/s while measuring the force. Short adhesion times were obtained by displacing the microsphere along the substrate at constant velocities v from 0.025 μm/s to 8.3 μm/s. The proteins on the sphere then interacted intermittently with the substrate, making the sphere roll. The time available for protein-substrate interaction was determined by the velocity v and the distance the microsphere rolled while a given protein was in proximity to the substrate, Δs (Fig. 1 *A*). Thus, the adhesion time was estimated as $\Delta t = \Delta s/v$. The rolling distance, Δs, was estimated from the width of force peaks, Δx, observed at high bond densities (Fig. 1 *B*), which corresponds to the diameter of the interaction area.

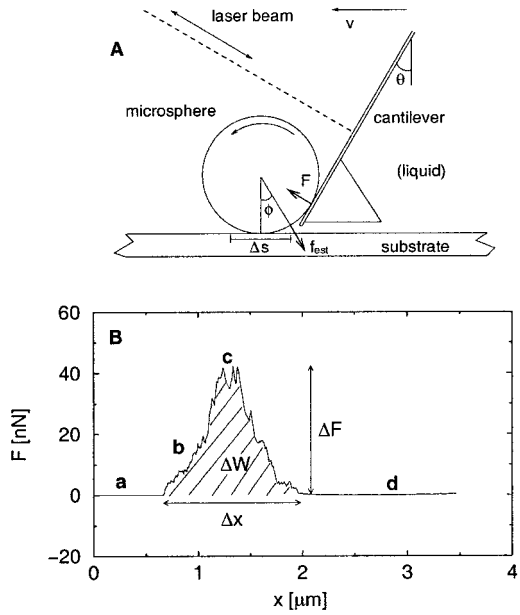

FIGURE 1 Microsphere displacement. (*A*) Each microsphere was displaced using an inclined AFM cantilever. Before displacement, the cantilever was aligned with the microsphere by use of an inverted optical microscope. The sample was then translated towards the cantilever at 0.83 μm/s. When interacting with the microsphere, the cantilever spring bent to apply a force to it, and the deflection was measured. To impose short adhesion times Δt on polystyrene, the microsphere was translated along the substrate at velocities v from 0.025 to 8.3 μm/s. Here, the adhesion time was estimated as $\Delta t = \Delta s/v$, where Δs is the distance a microsphere may roll while a given protein on the sphere surface is sufficiently close to interact with the substrate. The force acting on the microsphere from the cantilever, F, the force from a protein bond, f_{est}, the cantilever inclination to the vertical, θ, and the direction of f_{est}, ϕ, are also shown in this figure. (*B*) A force-distance curve. No force was recorded before the cantilever made contact with the microsphere (*a*). The force then increased (*b*) and protein bonds to the substrate ruptured (*c*). No force was recorded after all bonds had been removed (*d*). The baseline-to-peak force, ΔF, the peak width, Δx, and the work, ΔW, were found from the data.

Data analysis

Diode signal-versus-time curves were transformed to force-versus-displacement curves (Fig. 1 *B*), and filtered using a Wiener-Fourier filter attenuating frequencies above 45 Hz (Press et al., 1986). Force peaks were identified from the increased standard deviation of points within a sliding averaging window of 10 consecutive measurements. Peaks separated by <0.1 μm were treated as a single peak.

In the rolling technique used to obtain short adhesion times, the force was measured relative to a baseline value observed when the cantilever was not in contact with the microsphere. In these cases, the spheres reattached during displacement. Consequently, the force peaks could be arbitrarily wide, corresponding to several ordinary measurements made in series. To directly compare these measurements with those obtained by the other methods, the force-distance curves were sectioned in lengths equal to the estimated diameter of the sphere-substrate interaction area. Each section was then analyzed separately.

The peak with the largest area was selected as the peak of the sample. The baseline-to-peak force ΔF, the peak width Δx, and the work ΔW, were calculated (Fig. 1 *B*). The median was used to represent a group of observations, because this measure is still well-defined in cases where some measurements fall outside the dynamical range of the instrument.

To find peaks corresponding to single adhesion events, the data were filtered manually by interfacing the peak identification program to a plotting program. Force probability density distributions were estimated as follows: The N baseline-to-peak force observations were first sorted. The data were then binned in groups of m data points in increasing order, where the last bin could contain less than m points. The geometric mean, $\bar{Y}_i$, of the observations in bin i was calculated. To find the local density of data points, the width, Δ_i, of bin i was estimated as the sum of distances between the mid points of the mean observation, $\bar{Y}_i$, of the bin and the mean observations, $\bar{Y}_{i-1}$ and $\bar{Y}_{i+1}$, of the neighboring bins. The distances to the maximal and minimal observation were used at the ends of the distribution. The probability density was then estimated as $P(\bar{Y}_i) = m_i/(\Delta_i N)$.

RESULTS

Polystyrene substrate

The median adhesion force, ΔF, of microspheres to the hydrophobic polystyrene substrate is shown for short and intermediate adhesion times in Fig. 2. The observations were found to be consistent with the simple power-law expression

$$\Delta F = f_0\left(\frac{t}{t_0}\right)^a \quad (1)$$

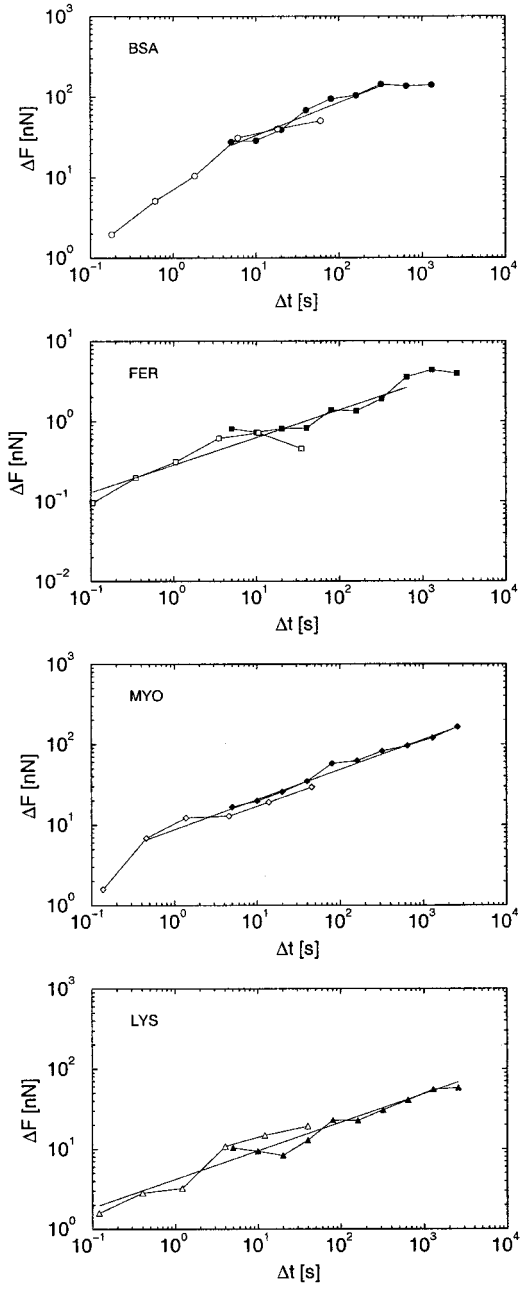

FIGURE 2 Adhesion force dynamics on polystyrene. The median adhesion force is plotted as a function of adsorption time. The closed symbols indicate samples where the adsorption time was controlled by first displacing the microspheres, waiting, and then measuring the force. The open symbols indicate cases where the microspheres were translated at constant speed while the instantaneous force was recorded. The adsorption time was then estimated from the displacement speed. The solid line shows a least-squares fit of the data to Eq. 1. Points at the ends were excluded from the fit if the saturation force had been reached or if a different scaling behavior was observed at small forces. The range of the fit is indicated by this line. The results and the rolling distances used for estimating the adsorption time are given in Table 1.

where f_0 is the force necessary to displace the microsphere at time $t = t_0$.

The parameters used and the results found by fitting the data to Eq. 1 are given in Table 1. The data collapse shown in Fig. 3 was obtained when normalizing these forces by f_0, setting $t_0 = 1$ s.

TABLE 1 Fitting parameters and results on polystyrene

Protein	Δs [μm]	f_0 [nN]	a
BSA	1.49	12.9 ± 1.8	0.41 ± 0.04
Ferritin	0.87	0.29 ± 0.04	0.34 ± 0.04
Myoglobin	1.1	8.7 ± 0.6	0.37 ± 0.01
Lysozyme	1.0	4.2 ± 0.4	0.36 ± 0.02

The rolling distance, Δs, over which a protein may interact with the surface may be estimated from the force peak width if the density of bonds to the substrate is high and the microspheres do not reattach when displaced. The force peak widths were therefore found using data from an earlier experiment (Sagvolden et al., 1998) where the surface was blocked to prevent reattachment and bond density was high. However, reattachment rates were considerable for lysozyme and myoglobin, and the peak widths were therefore estimated from observations of individual peak shapes in these cases. The force at $t = t_0 = 1$ s, f_0, and the exponent a were found by a least squares fit of the data to Eq. 1, and are given together with the standard error of the fit.

Glass substrate

The forces after intermediate adhesion times on glass are shown in Fig. 4 *A*. Fewer than 50% of the BSA- or ferritin-coated spheres adhered with an observable force; thus, the median force was 0 pN. The median force of the myoglobin sample increased with the adsorption time, whereas the forces measured on the lysozyme sample were constant. Hence, the time to reach the saturation force was <5 s for lysozyme and >1280 s for myoglobin. The typical time to form a bond was >1280 s for BSA and ferritin.

The force distribution moved towards larger forces for the BSA and ferritin samples even though the median force was 0 pN. This is shown in Fig. 4 *B*, where force at the 80th percentile is plotted. This force, at the high end of the distribution, was nonzero and increased with time for both samples.

An increased number of adsorbed proteins does not necessarily lead to a proportional increase in force because the

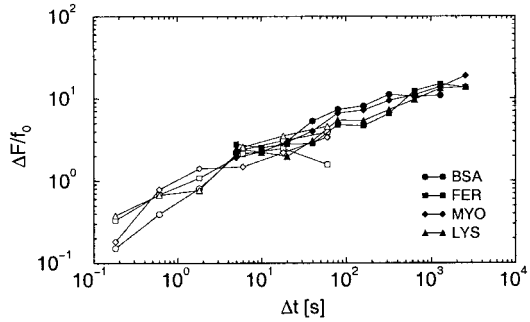

FIGURE 3 Data collapse for protein adhesion forces on polystyrene. The adhesion forces shown in Fig. 2 were divided by f_0, the force at $t = t_0 = 1$ s, found by a fit of Eq. 1 to the data in Fig. 2. This shows that the time course of protein adhesion on the hydrophobic surface may be characterized by a common exponent, $a = 0.37 \pm 0.03$, and a protein-dependent force amplitude, f_0.

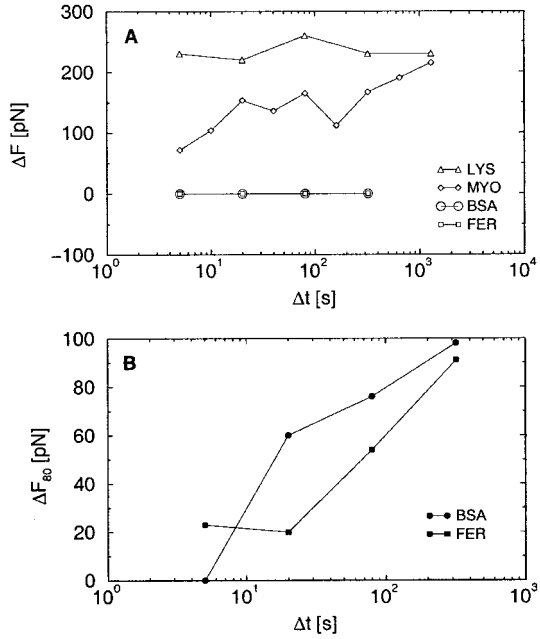

FIGURE 4 Short adhesion times on glass. Adhesion forces on glass were measured by first displacing the microsphere, waiting, and then measuring the force. In *A*, the median force is shown as a function of adsorption time. Adhesion forces were observed for less than 50% of the ferritin and BSA samples. In *B*, the force at the 80th percentile is plotted, showing that the force distribution moved towards higher forces when the adsorption time increased.

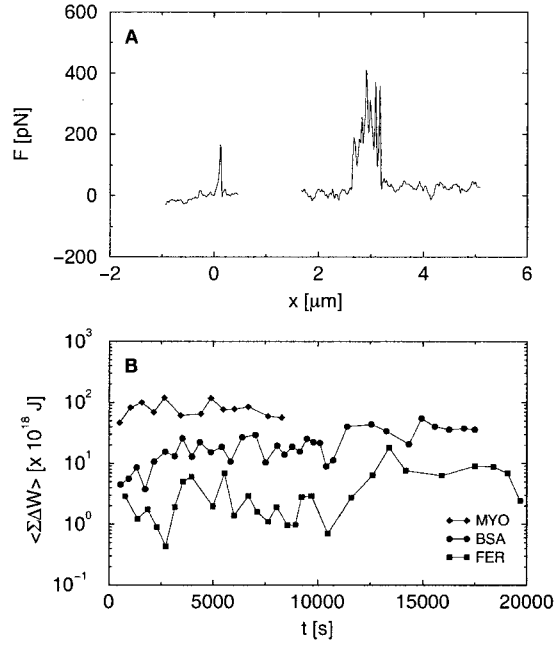

FIGURE 5 Time-dependent adhesion on glass. The number of bonds formed to the substrate increased with adhesion time. In (*A*), force-distance curves for myoglobin are shown. The curve to the left is typical for small adhesion times, whereas the curve to the right represents a longer adhesion time. The latter is a superimposition of several peaks, each due to an adhesion event. As the difference in maximal force of these curves is small compared to the difference in their area, the time dependence of adhesion is found form the median work to remove the microspheres. (*B*) The median work of 19 consecutive measurements is shown as a function of the mean time since the microspheres were seeded on the substrate. The myoglobin sample reached equilibrium in $<$1000 s and the BSA sample in about 3000 s, whereas the saturation time for the ferritin sample was $>$10,000 s. The lysozyme sample reached equilibrium in $<$5 s and is not included in this figure.

bonds to glass substrates are often sparsely distributed and not stressed simultaneously (Fig. 5 *A*). However, the total work, $\Sigma\Delta W$, increases with the number of bonds, and was therefore used to estimate the time used to reach equilibrium adhesion. As shown in Fig. 5 *B*, the myoglobin sample reached equilibrium in about 1000 s, BSA in 3000 s, and ferritin in $>$10,000 s.

Single protein adhesion forces

Forces due to single adhesion events could be identified in the observations of adhesion to the glass substrate because few bonds were formed in this case. To identify these forces, observations consisting of a single peak (Fig. 5 *A*, *left*) were used, excluding observations with multiple peaks (Fig. 5 *A*, *right*). These peaks were all narrow, consistent with the idea that they represent situations where the bonding proteins are stretched in parallel.

The probability density distributions of the force data (Fig. 6) were used to identify forces due to a single adhesion event, f_1, two adhesion events, f_2, and so on. The mean force, f_i, the mean work, w_i and the mean peak width, Δx_i were calculated for each peak in the distributions (Table 2).

For BSA, peaks at $f_1 = 122 \pm 25$ pN and at $2f_1 \simeq f_2 = 232 \pm 24$ pN were observed. The higher order peaks were at lower forces than integer multiples of f_1, which may be explained since a microsphere held by a single or two bonds may rotate to distribute the forces to the bonds, while movement is restricted if it is held by three or more bonds. Consequently, the load is not equally distributed and the maximal force is reduced while the peak width increases.

The first peak in the force distribution for the myoglobin sample appears at $f_1 = 88 \pm 15$ pN. The second, at $f_2 = 177 \pm 28$ pN, is a multiple of this force. If the bonds to the surface were equal, as assumed in this analysis, the work done to displace the microsphere is a multiple of the work to displace a single bond. This is the case for the first and second peak in the distribution, at $w_1 = 2.5 \pm 1.0$ aJ and $w_2 = 5.4 \pm 1.9$ aJ, but the mean work in third and fourth peak is close to 4 and 6 times w_1 respectively. This indicates that force peaks corresponding to 3 and 5 adhesion events were not identified in this sample. If this is the case, then the observed forces also agree with the observations for BSA, where the third and higher order forces were less than an integer multiple of the single event force.

In the ferritin sample, it is suspected that the peak corresponding to a single adhesion event partly disappears in the noise. The separation of the subsequent peaks indicate that the force f_2 corresponds to two adhesion events. The force per adhesion event was therefore estimated as $f = f_2/2 = 29$

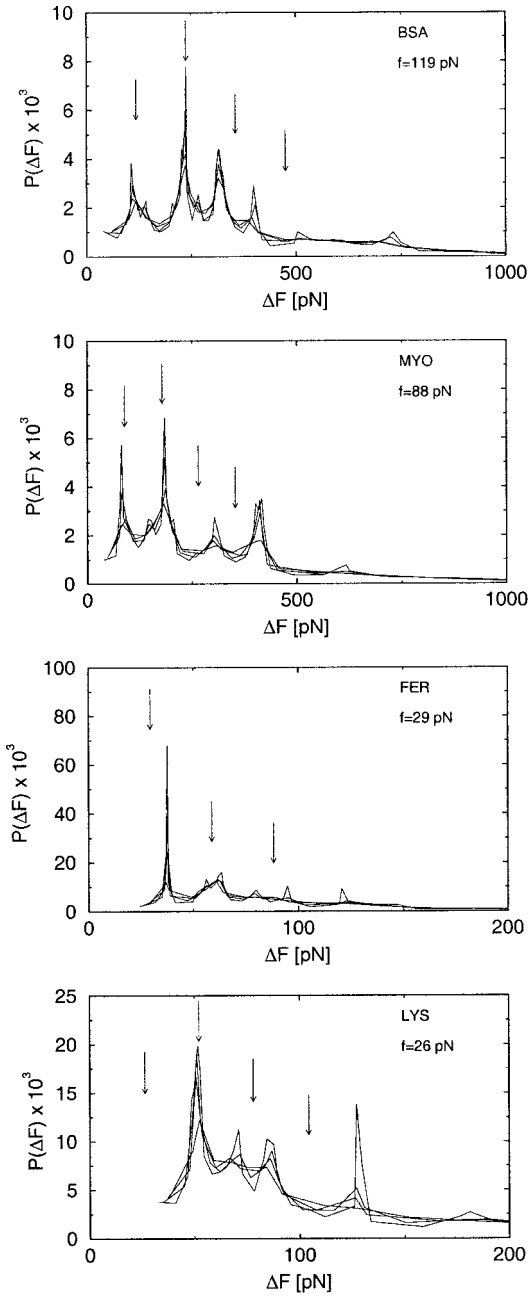

FIGURE 6 Force probability density distributions. The data were filtered to identify single force peaks corresponding to the one in Fig. 5 *A*, *left.* Peaks with shapes corresponding to Fig. 5 *A*, *right*, were discarded, since these multiple adhesion events cannot easily be deconvoluted. Probability density distributions are shown for bin sizes m of 2, 3, 4, and 5 observations (see Materials and Methods). The mean force, work, and force peak width were calculated from the force-distance curves of the samples belonging to each peak in the probability density distributions (Table 2). The arrows correspond to integer multiples of the single adhesion event force, f.

pN, and not the average of f_1 and f_2 weighted by the number of observations n, as in the BSA and myoglobin samples.

Estimates of the single event adhesion force are difficult to make on the lysozyme sample. Although force peaks appear in the distribution, their separation indicates that the first observed peak corresponds to adhesion of more than a single bond. This sample reached saturation in <5 s, but at low forces (Fig. 4 *A*). Thus, lysozyme adheres quickly, but at a low force per molecule. The data did, however, allow for an estimate of the single bond force. The first peak was observed at 52 ± 5 pN. Because the force increments between subsequent peaks were 17 pN on average, and the separation in force between the two first peaks was slightly larger than that between the subsequent ones in the other samples, it was inferred that the first peak in the distribution corresponds to two adhesion events. If this is the case, the force per lysozyme adhesion event is $f = f_1/2 = 26$ pN. The data on the average work, w_i, support this conclusion.

The manipulation force microscope measures the forces exerted by the cantilever on the microsphere (Fig. 1 *A*). The force on the protein bonds depends on geometric factors, such as the direction of applied force, the microsphere radius, and the bond length (Chang and Hammer, 1996). This force may be estimated using a force-balance model (Sagvolden et al., 1999a). Assuming that a microsphere is held by a single protein and that frictional forces are negligible, the maximum force on the protein, f_{est}, is

$$f_{est} = \frac{\cos\theta}{\sin\phi}F \simeq \frac{\sqrt{3}/2}{\Delta s/(2R)}F = \frac{\sqrt{3}R}{\Delta s}F, \qquad (2)$$

where $\theta = 30°$ is the direction of the radial force applied by the cantilever on the microsphere, ϕ is the direction of the force from the protein, Δs is the diameter of the protein-substrate interaction area, R is the microsphere radius, and F is the maximum force on the cantilever. The estimated protein forces are given in Table 3.

The adhesion forces between the cantilever surface and the microspheres were not measured directly in the experiment. However, it was observed that BSA- and ferritin-coated microspheres, in contact with both the substrate and the cantilever, always adhered to the substrate when the cantilever was removed. Thus, the microspheres had a stronger affinity for the substrate. The myoglobin- and lysozyme-coated microspheres had an observable affinity for the cantilever surface. This affinity was reduced, but not eliminated, by displacing the microspheres using a protein covered surface. Thus, the friction between the cantilever and the microsphere may not have been negligible in the latter case.

DISCUSSION

I am not aware of any other study measuring the adhesion forces of proteins to substrates as a function of time or attempting to identify the adhesion force of individual proteins.

In a previous study (Sagvolden et al., 1998), it was shown that protein-covered microspheres adhere with forces characteristic of the protein and substrate. It was proposed that the force is determined mainly by the ability of the molecules to change conformation on the hydrophobic polysty-

TABLE 2 Single adhesion events on glass

Protein	i	n	f_i [pN]	w_i [aJ]	Δx_i [nm]
BSA	1	20	122 ± 25	4.7 ± 2.3	118 ± 32
BSA	2	26	232 ± 24	9.8 ± 4.2	147 ± 44
BSA	3	22	321 ± 21	14.7 ± 3.8	175 ± 32
BSA	4	10	403 ± 11	17.9 ± 4.0	180 ± 17
BSA average			119 ± 19	4.8 ± 1.8	
Myoglobin	1	13	88 ± 15	2.5 ± 1.0	86 ± 26
Myoglobin	2	24	177 ± 28	5.4 ± 1.9	99 ± 29
Myoglobin	4	14	296 ± 27	10.7 ± 3.6	116 ± 36
Myoglobin	6	15	405 ± 21	15.4 ± 4.2	127 ± 35
Myoglobin average			88 ± 14	2.6 ± 0.9	
Ferritin	1	15	36.3 ± 3.2	0.75 ± 0.12	60 ± 13
Ferritin	2	20	58.7 ± 5.6	1.37 ± 0.60	68 ± 23
Ferritin	3	9	78.8 ± 3.9	1.87 ± 0.92	75 ± 29
Ferritin estimate			29.4 ± 2.8	0.69 ± 0.25	
Lysozyme	2	17	52.3 ± 5.4	1.11 ± 0.30	63 ± 14
Lysozyme	3	9	70.1 ± 3.3	1.39 ± 0.49	56 ± 11
Lysozyme	4	10	86.7 ± 4.0	1.96 ± 0.22	59 ± 4
Lysozyme	6	8	129.1 ± 3.1	2.94 ± 0.53	69 ± 17
Lysozyme estimate			26.2 ± 2.7	0.51 ± 0.13	

The mean force, f_i, the mean work, w_i, the mean width of the peak in the force distance curves, Δx_i, and the standard deviations were calculated for each peak identified in the force probability density distributions (Fig. 6). These peaks were assumed to correspond to integer number of bonds, i, being stretched concurrently. To estimate the force per adhesion event in the BSA and myoglobin samples, the mean force, f_i, corresponding to 1 and 2 bonds was weighted with the number of observations, n, whereas it was estimated as half the force corresponding to two adhesion events in the ferritin and lysozyme samples. The average work was found by the n-weighted average of all identified force peaks.

rene substrate, while protein charge constitutes a barrier to adsorption on glass.

Here, it is shown that the force exerted on glass per adhesion event is larger for BSA and myoglobin than for ferritin and lysozyme. This force ranking corresponds well to the relative stiffness of the proteins, where the BSA and myoglobin are structurally softer than ferritin and lysozyme (Harrison, 1959; Tripp et al., 1995). Hence, structural changes seem to influence the adhesion force on glass. Changes in protein structure upon adsorption to glass have indeed been observed by others (Kondo et al., 1991; Norde and Favier, 1992; Haynes and Norde, 1995).

However, the primary factor determining the force necessary to displace the microspheres on glass is the number of bonds to the substrate. Even if BSA adheres with a large force, the number of bonds is small compared to that for lysozyme- or myoglobin-coated microspheres. This is due to a barrier to adsorption, which correlates well with the net protein charge, in agreement with earlier results (Sagvolden et al., 1998).

TABLE 3 Estimated forces acting on a bond

Protein	Δs [μm]	f_{est} [pN]
BSA	1.49	277 ± 44
Myoglobin	1.1	277 ± 44
Ferritin	0.87	115 ± 13
Lysozyme	1.0	90 ± 10

The force acting on a protein bond, f_{est}, was estimated from the results in Table 2 using Eq. 2. The same rolling distance, Δs, as on the polystyrene surface was used.

The width of the peaks in the force-distance curves suggests that the proteins unfold under strain. Hence, the work done when displacing the microspheres have contributions from the energy to denature the proteins and the energy to remove them from the surface. The protein denaturation energies may be measured by microcalorimetry for single-domain proteins such as myoglobin and lysozyme. The free energy of denaturation is 0.096 aJ for lysozyme and 0.041 aJ for myoglobin at 25°C in aqueous solution (Makhatadze and Privalov, 1995). These energies account for only a small amount of the work done. Hence, most of the work is expended to remove the protein from the substrate.

A power law dependence of the median adhesion force with time on polystyrene indicates that the adhesion process is fractal. This implies that this sample has no typical, well-defined time scale for adhesion, but adhesion times varying over many orders of magnitude.

The exponent $a = 0.37 \pm 0.03$ was similar for all the proteins tested. Hence, the time dependence of the protein adhesion force on polystyrene may be characterized by this common exponent and a protein-dependent force amplitude, f_0.

The samples have an asymptotic adhesion strength because the number of proteins that may bind a single microsphere is limited. The observations indicate that the BSA sample reached saturation at long adhesion times. Hence,

the adhesion force on polystyrene may be characterized by a power law at short times, crossing over to a constant force at long adsorption times.

These samples exhibit large differences in the force amplitude, f_0, that correlate well with protein stiffness (Harrison, 1959; Tripp et al., 1995). Hence, the protein's ability to undergo structural changes is important in deciding the adhesion force, as concluded in an earlier study (Sagvolden et al., 1998).

The adhesion forces measured in the previous experiment (Sagvolden et al., 1998) were reproduced qualitatively, but with some differences in the absolute forces obtained. This may be due to differences in the details of the experiments, because protein was added to the buffer in the first experiment, blocking adhesion between the cantilever and microsphere, and the pH was slightly lower. These deviations were largest for the myoglobin and lysozyme samples on the glass substrate. These microspheres were sticky and had some affinity for the cantilever. Thus, the friction against the cantilever may not have been negligible in these two samples, which may affect the measured adhesion force.

In conclusion, the adhesion force of microspheres bound by proteins to polystyrene and glass substrates was measured over several orders of magnitude in time. The adhesion force followed a power-law behavior on polystyrene. On glass, forces due to single adhesion events were identified.

This work was supported by a grant from the Norwegian Research Council. I am also indebted to Jens Feder and Ivar Giaever for helpful discussions and advice.

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