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Nonlinear Kinetics of Ferritin Adsorption

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ABSTRACT The adsorption of ferritin at a methylized quartz surface was measured with off-null ellipsometry and transmission electron microscopy. An initial lag-phase was seen, followed by an accelerating adsorption leading to mass transport limitation of the reaction. The rate of adsorption then decreased at a surface concentration far below monolayer coverage, and a continuously decreasing rate of binding was seen. The slope of the binding rate was linear with the logarithm of time (fractal kinetics). The adsorbed ferritin molecules were distributed in clusters as seen by transmission electron microscopy. Clusters grown during the mass transport limited adsorption had crystalline structure at short range and low fractal dimensions ($d_f = 0.89$) over long range. Clusters grown during adsorption with fractal kinetics showed random structure at short range and a high fractal dimension $d_f = 1.86$ over all ranges. These findings indicate some new important mechanisms responsible for the complex kinetics of macromolecular reactions at solid-liquid interfaces. The results are discussed in relation to recently developed theories of self-organized criticality.

INTRODUCTION

Macromolecular reactions at liquid-solid interfaces have several chemical characteristics in common. The initial reaction of enzyme-substrate interactions (Trurnit 1954), antigen-antibody reactions (Nygren and Stenberg 1985), and protein adsorption (de Feijter et al. 1978) often becomes mass transport limited. Measurements of the reaction kinetics are hampered by the effect of mass transport limitation, which is due to the depletion of ligand in the reaction zone close to the surface, and no conclusions can be made from such measurements regarding the sticking probability at the surface. This is a serious shortcoming of experimental data that hinders the development of theoretical models of macromolecular reactions at interfaces. In the present study precautions were taken to avoid mass transport limitations by using a flow cuvette and fast ellipsometric detection.

Under these conditions, mass transport limitation is described by the relationship (Stenberg and Nygren 1988)

$$dS/dt = (DC_0)/R \qquad (1)$$

where S is the surface concentration of adsorbed protein, D is the diffusion constant of the protein, C_0 is the concentration of protein in the solution, R is the thickness of the unstirred water film, and t is time.

After the diffusion limitation the reaction rate decreases logarithmically over long periods of time as shown for protein adsorption (Cuypers et al. 1987) and antigen-antibody reactions (Nygren and Stenberg 1989; Werthén et al. 1990).

The present study was undertaken in order to describe the molecular mechanism behind the kinetics of macromolecular reactions at interfaces. Adsorption of ferritin at a hydrophobic surface is a suitable model system with the characteristic kinetics described above (Nygren and Stenberg 1990). The iron nucleus of ferritin makes it possible to use transmission electron microscopy to study the spatial distribution of ferritin over time during the process of adsorption.

MATERIALS AND METHODS

Chemicals

Ferritin crystallized three times (Fluka Chemie AG, Buchs, Switzerland) was dissolved in 0.05 M phosphate buffer (pH 7.2) containing 0.15 M NaCl. Hexamethyldisilazane (E. Merck, Darmstadt, Germany) was used for methylization of SiO_2 surfaces (oxidized silicon wafers) as described previously (Jönsson et al. 1985).

Ellipsometry

A null ellipsometer (Rudolph Research model 436) was used with a He-Ne laser (632.8 nm) as the light source. The angle of incidence was 70°. A 50-μl flow cuvette (Hellma, Müllheim, Germany) was used, with the back wall comprising the exchangeable silicon substrate. Phosphate buffer solution (0.05 M, pH 7.2) containing 0.15 NaCl was pumped through the cuvette at a rate of 3 ml/min. The general procedure followed was to first find the polarizer and analyzer positions resulting in a minimum of light transmission through the instrument (null ellipsometry). Any change in the thickness d or refractive index n of the sample surface will result in an increased light intensity I passing through the instrument. The relation can be written (Stenberg and Nygren 1983)

$$I = k(n,d_0)d^2 \qquad (2)$$

where k is a constant for a given sample and can be seen as a sensitivity constant since it is a function of all optical parameters involved. In the present study the refractive index of the film n and the initial film thickness d_0 are the parameters that determine the sensitivity. The refractive index of ferritin was independently determined to be 1.7 by using an Abbe refractometer on ferritin solutions.

Protein solution was then pumped through the cuvette and the change in the light intensity was recorded.

At a steady state of adsorption, new positions of the polarizer and analyzer giving a minimum of light transmission were sought in order to determine the change in the ellipsometric readings due to the adsorbed protein layer. The silicon wafers used in the present study had a thin (2 nm) SiO_2 film. For such surfaces there are only small changes in the analyzer position due to protein adsorption. Since the refractive index of the film is known

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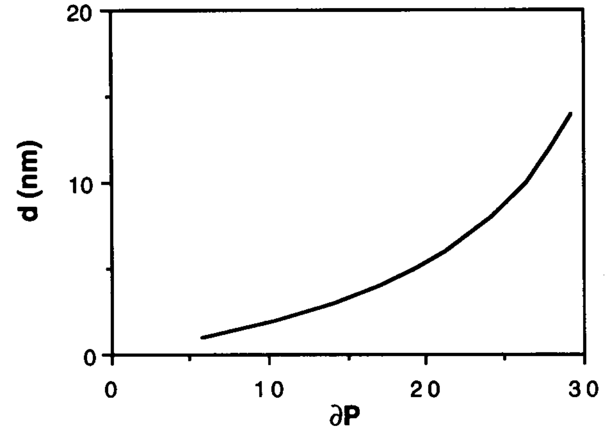

FIGURE 1 Calculated change in polarizer setting δP from the null position in null ellipsometry versus thickness of an adsorbed film with a refractive index of 1.7. The substrate is an oxidized (2-nm oxide thickness) silicon wafer with a complex refractive index of 3.858-i0.018 at the wavelength 632.8 nm. The refractive index of the oxide is 1.458, and the angle of incidence is 70°.

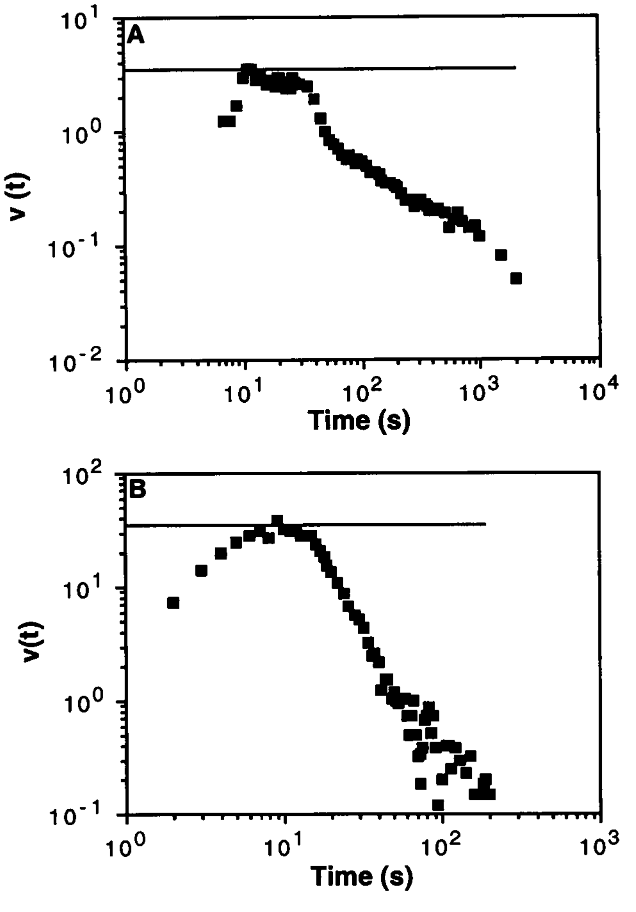

FIGURE 2 The velocity of ferritin adsorption (ng cm^{-2} S^{-1}) onto a hydrophobic surface plotted versus reaction time (S) as measured by off-null ellipsometry with a time resolution of 2 Hz. Solid line, mass transport-limited adsorption over a 10-μm-thick dead layer. (*a*) concentration of ferritin in the solution = 10 μg/ml. (*b*) concentration of ferritin in the solution = 100 μg/ml.

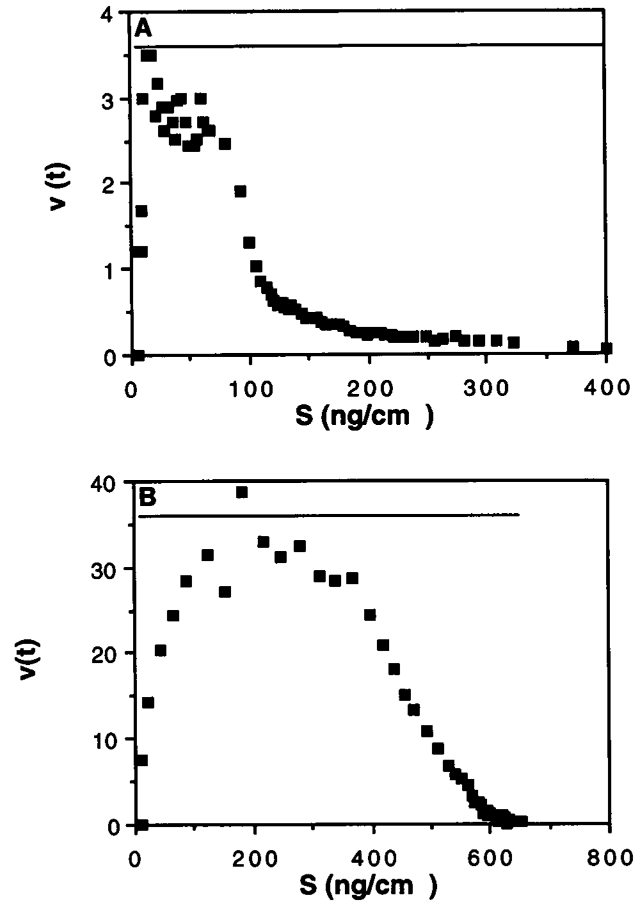

FIGURE 3 A plot of the velocity versus surface concentration of ferritin adsorption onto a hydrophobic surface measured by off-null ellipsometry with a time resolution of 2 Hz. Solid line, mass transport-limited adsorption over a 10-μm-thick dead layer. (*a*) concentration of ferritin in the solution = 10 μg/ml. (*b*) concentration of ferritin in the solution = 100 μg/ml.

independently we can thus use a single relation between protein film thickness and change in polarizer position as shown in Fig. 1 in order to calibrate the light intensity versus film thickness. The value of the film thickness is inserted in Eq. (2) together with the measured off-null intensity, and the constant k is obtained.

Due to stray light and imperfections there is always a small residual intensity I_0 at null settings. This value is simply subtracted from the intensity. The film thickness during adsorption is then given by

$$d = k'\sqrt{I - I_0} \tag{3}$$

The thickness d is an equivalent optical thickness. The relation between this film thickness and the amount of bound protein can be found in different ways, depending on which properties of the protein are most well characterized experimentally. With a well-known density of the protein, the relation

$$\Gamma = d\zeta \times 100 \tag{4}$$

can be used, where Γ is the surface concentration (ng/cm^2), d is film thickness (nm), and ζ is the density of the protein (g/cm^3). The density of the ferritin was determined pycnometrically as described (Bernhardt and Pauly 1980) to be 1.6 g/cm^3.

Electron microscopy

Grids with an electron transparent film of SiO_2 where produced and methylsilanized as described (Stemme et al. 1987). The sample grids

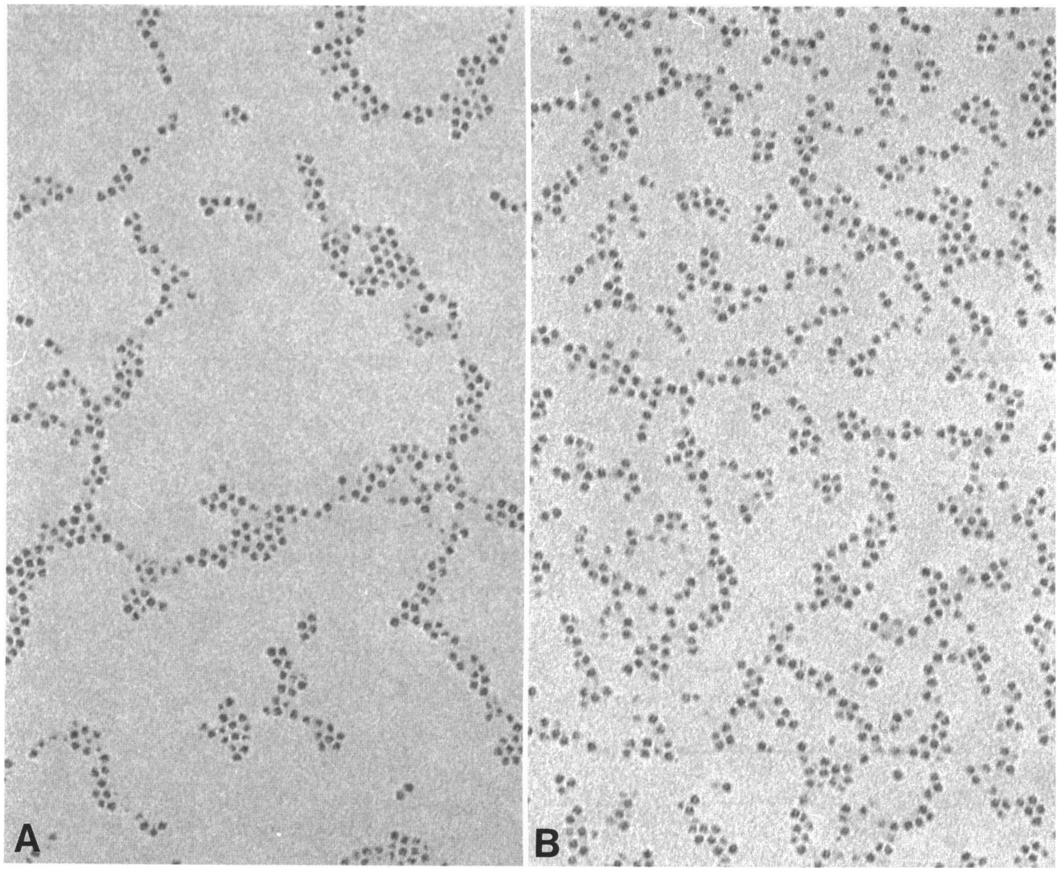

FIGURE 4 Electron micrograph of the spatial distribution of adsorbed ferritin. (*a*) adsorption time 40 s from a concentration in solution of 10 μg/ml. (*b*) adsorption time 1800 s from a concentration in solution of 10 μg/ml.

were exposed to ferritin as described previously (Nygren and Stenberg 1990) and examined in a Philips 400 electron microscope. Micrographs were taken at a primary magnification of ×20,000 and were further magnified as copies.

Image analysis

The fractal dimension and the radial distribution function $g(r)$ of adsorbed ferritin were evaluated from the distribution of molecules as a function of the radial distance from a given molecule $M(r)$, using a circular grid. The slope of log $M(r)$ versus log (r) determines the fractal dimension d. The radial distribution function $g(r)$ was obtained by relating the distribution of molecules to a random distribution by normalizing with the density probability

$$A^{-1}[N(N-1)]^{0.5} \qquad (5)$$

where A is the area and N the number of molecules.

RESULTS

The rate of adsorption of ferritin (ng cm^{-2} S^{-1}) versus adsorption time (S) is shown in Fig. 2. An initial acceleration of adsorption is seen for both protein concentrations. The rate increase is damped by mass transport limitation. The mass transport limited adsorption is followed by a logarithmic decrease of the adsorption rate. The slope of the logarithmic rate decrease is $t^{-0.67}$ for a bulk concentration of 10 μg/ml (Fig. 2 *a*) and $t^{-2.48}$ for a bulk concentration of 100 μg/ml (Fig. 2 *b*). A similar plot of the rate of adsorption versus the surface concentration of adsorbed protein is shown in Fig. 3. The retardation of adsorption is seen at different surface concentrations for the two bulk concentrations used. Thus the rate decrease is not due to a simple surface exclusion effect.

Electron micrographs of the distribution of adsorbed ferritin during the mass transport limited and logarithmic phases of adsorption are shown in Fig. 4. The protein is distributed as clusters during the whole process of adsorption. During mass transport limited adsorption, the structure of the clusters is orderly, with clearly distinguished crystalline areas (Fig. 4 *a*). During the logarithmic phase of binding, the clusters are less orderly, but linear arrays of molecules are frequently found (Fig. 4 *b*).

The adsorbed clusters are fractals, as shown in Fig. 5 *a*. The fractal dimension of the clusters is not stable during the adsorption process but increases during the period of logarithmic decrease of the rate of adsorption. Clusters grown during mass transport limitation have a low fractal dimension of 0.89 (regression coefficient = 0.99) at distances of >3 molecular radii, whereas clusters formed during the logarithmic decrease have a high fractal dimension of 1.86 (r = 1.0). The radial distribution function $g(r)$ of the distribution of adsorbed ferritin is shown in Fig. 5 *b*. The distribution of molecules in clusters grown under mass transport limitation

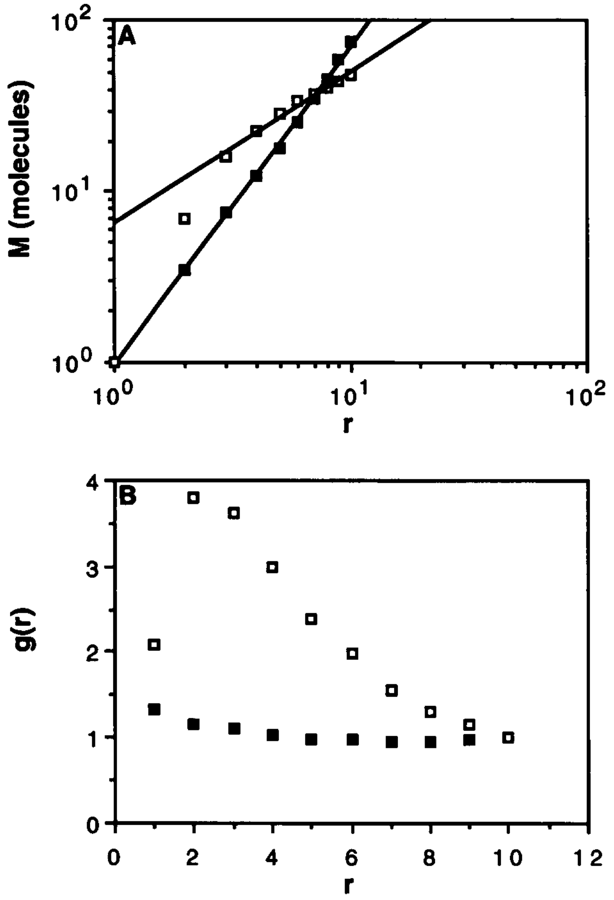

FIGURE 5 Radial distribution of ferritin measured from pictures shown in Fig. 4. (*a*) fractal dimension of ferritin aggregates obtained from a log-log plot of the radial distribution of molecules. □, data from Fig. 4 *a*; ■, data from Fig. 4 *b*. (*b*) normalized radial distribution function $g(r)$ of adsorbed ferritin. □, data from Fig. 4 *a*; ■, data from Fig. 4 *b*.

shows an increased probability of finding a neighbor at distances less than 6 radii. In clusters grown during the logarithmic decrease of the adsorption rate, the radial distribution function is close to 1, indicating a nearly random distribution of adsorbed molecules.

DISCUSSION

The kinetics of protein adsorption was measured with off-null ellipsometry in situ allowing a time resolution of 0.1 s, which makes it possible to measure the initial adsorption taking place before the diffusion layer in the solution is depleted of protein.

The acceleration of the initial adsorption can be described by nucleation-and growthlike kinetics assuming attraction between adsorbed molecules and molecules in solution. The initial cooperative adsorption can be described theoretically by an exponential growth (Nygren et al., 1993), which rapidly leads to depletion of reactants in the reaction zone, making the reaction mass transport limited.

The mass transport limitation shown in Fig. 2 is based on the assumption that the thickness of the unstirred layer is 10 μm. This assumption seems reasonable for a well stirred bulk phase obtained by using a flow cuvette with a small volume, turbulent flow, and a high flow rate and a continuous exchange of the bulk phase.

The clusters formed during mass transport limited adsorption have been computer simulated using a two-dimensional stochastic cellular automaton (Stenberg and Nygren 1991). The experimental patterns of clusters that are dense at short ranges and show low fractal dimensions at long ranges can be simulated by assuming growth mechanisms, including lateral interactions giving rise to phase separations in the adsorbed layer and dissociation from dense cluster regions. Similar findings of two regimes of growth kinetics and increasing fractal dimensions of surface aggregates have been reported by Schwartz et al. (1992).

The logarithmic decrease of the adsorption rate seen in Fig. 2 remains to be explained. The general phenomenon of logarithmically decreasing reaction rates has been collectively named "fractal kinetics" (Kopelmann 1986) and has been demonstrated experimentally in a number of situations (Kopelmann 1988). Spatial and/or energetic heterogeneity of the medium or nonrandomness of the reactant distribution in low dimensions have been suggested as mechanisms behind the phenomenon fractal kinetics. Theoretical consideration raised by statistical methods of random walk on fractal structures show that fractal kinetics is not due to the number of binding sites on each protein molecule but is a reflection of the fractal properties of proteins and enzymes (Li et al. 1990).

In the present study, the main finding is that ferritin clusters are restructured during adsorption with fractal kinetics. Orderly, structured aggregates seen in previous phases of adsorption disappear, and a more random distribution is seen. This process of rearrangement of dense clusters is not necessarily a continuous one but may take place through critical dissociation of orderly structured aggregates as suggested previously (Nygren 1992). An intellectual model of this phenomenon is the continuous build-up and discontinuous fall of sand on sandpiles in an hourglass. Theoretical models of such processes have been elaborated on (Bak et al 1988; Bak and Chen 1989) and may serve to explain the fractal kinetics of macromolecular reactions at surfaces. The use of such models has been suggested for intramolecular protein dynamics (Dewey and Bann 1992).

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