Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 5557–5561, July 1991
Biochemistry

Adsorption/desorption of human serum albumin on hydroxyapatite: A critical analysis of the Langmuir model

(adsorption kinetics/isotherm/random sequential adsorption)

M. J. MURA-GALELLI*, J. C. VOEGEL*, S. BEHR*, E. F. BRES*, AND P. SCHAAF†

*Centre de Recherches Odontologiques, U157 Institut National de la Santé et de la Recherche Médicale, ULP 1, Place de l'hopital, 67000 Strasbourg, France; and †Institut Charles Sadron (Centre National de la Recherche Scientifique-ULP), 6, rue Boussingault, 67083 Strasbourg, France

Communicated by Howard Reiss, March 25, 1991 (received for review October 2, 1990)

ABSTRACT **We studied the adsorption of human albumin onto synthetic hydroxyapatite, using a radiotracer technique and a special flow cell. Adsorption was studied under various conditions corresponding to different thermodynamic paths. It appears that (*i*) as is the usual case, the isotherms obtained within a short time range (a few hours) do not correspond to a true equilibrium situation; (*ii*) when the adsorption process is followed for longer times, which is necessary at low bulk concentrations, one always reaches the plateau surface adsorption; (*iii*) this plateau value is independent of the "history" of the adsorption process and corresponds well to the jamming limit predicted by the random sequential adsorption model; and (*iv*) surface denaturation, leading to enhanced surface binding and thus decreasing desorption constants, is the important phenomenon that can partly and qualitatively explain our observations. Its time dependence, however, remains to be clarified.**

The adsorption of proteins from solution onto solid surfaces is a fascinating and complex process that is known to have great biological impact (1). It exhibits both reversible and irreversible aspects, which often seem to be in mutual contradiction. For example, adsorbed proteins can be exchanged with similar or different proteins from the bulk solution (2–5). However, on the time scale of the experiments, only a limited population of adsorbed molecules seems to be "exchangeable." In addition, when a protein solution in contact with a solid phase is suddenly replaced by buffer, generally only a small fraction of the adsorbed molecules are desorbed (3, 5). Moreover, it has progressively been recognized that the irreversible aspects of these processes increase significantly with the mean contact time between the proteins and the adsorbing surface (6, 7). This is explained by interfacial molecular denaturation, leading to stronger interactions between the adsorbate and the surface. The observations of Jennissen and Botzet (8, 9), who found desorption hysteresis in the phosphorylase b_c/butyl-Sepharose system, have been interpreted in this way.

Another typical consequence of the apparent and partial "irreversibility" of the adsorption/desorption process is that the amount adsorbed is not a unique function of the concentration of the solution in "equilibrium" with the surface; the "history" of the process plays a significant role. When adsorption is performed in one step, with the surface brought directly into contact with a solution of bulk concentration c_b, the amount adsorbed is different (often higher) than in the case of a step-by-step experiment (the bulk concentration is then increased stepwise up to c_b).

The purpose of the present paper is to go one step further in the analysis of this puzzling phenomenon of protein adsorption. Two questions will be addressed in the discussion of our experimental results. (*i*) Is it still possible to speak about "isotherms" and surface/solution "equilibrium" when dealing with some protein/adsorbent systems? (*ii*) Does one usually take into account the appropriate characteristic time scales that govern the adsorption process under study?

We chose to study the adsorption of human serum albumin onto synthetic hydroxyapatite. This adsorbent is often used for chromatographic purposes (10–12), and biological apatites are major constituents of hard tissues, bones, and teeth. In addition, apatites also possess good biocompatibility (13) when exposed to the numerous proteins dissolved in the body fluids (bone mineral) or in saliva (dental enamel). The surface is then quickly covered by a proteinaceous layer. Among these proteins, albumin is dominantly present.

This article follows previous work (14–16) where kinetic aspects were investigated, for the apatite/albumin system, in what will be called the short time range, in experiments lasting less than a few hours. The experimental setup, which uses protein labeling, was designed as a "chemical reactor." The adsorption process may be followed continuously as a function of time, the bulk concentration (and thus the history of the process) being varied at will, over very different time periods. Such an experimental procedure is particularly adapted to study adsorption on finely divided matter and could also be transposed to biological cells, which should not be altered by this particular environment.

MATERIALS AND METHODS

Adsorbent. Synthetic hydroxyapatite powder (Bio-Gel HTP, Bio-Rad) was synthesized as described by Tiselius *et al.* (17). It was sieved several times (Pulverisette, Sodilab, Strasbourg, France) in order to recover the 90- to 150-mesh (100- to 160-μm) sample having a specific area of 45 $m^2 \cdot g^{-1}$, as measured by N_2 adsorption. The sample was then washed with CH_3OH and dried at 40°C for 24 hr.

Adsorbate. Human serum albumin (M_r 68,000) provided by the Centre National de Transfusion Sanguine (Strasbourg, France) was radiolabeled with ^{125}I by the ICl method (18). The concentration of the protein solution was measured by absorbance at 280 nm, using an extinction coefficient of $3.604 \times 10^7\ M^{-1} \cdot cm^{-1}$ (19), and its specific activity ($cpm \cdot mg^{-1}$) was established by γ counting (Packard, United Technologies). The protein was dissolved in a 50 mM Tris·HCl/150 mM NaCl/1 mM NaN_3, pH 7.35.

Experimental Apparatus and Procedure. A special flow cell has been designed (20) in which the dispersed adsorbent material is in direct contact with a well-stirred solution containing the albumin adsorbate. The cell is thermostatted at 27°C, its volume is 13.3 cm^3, and it contains about 50 mg of hydroxyapatite powder, so that this adsorbing surface is greatly superior to that of the internal cell and tubing surfaces, which, in addition, are treated separately to make them nonadsorbant with respect to proteins (21).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

When a mass balance equation is applied between initial and final states of the system, the amount of albumin adsorbed is easily calculated. However, the main advantage of our experimental setup, which contains an inlet and an outlet for the passage of protein or buffer solutions, is that it enables real-time evaluation of the amounts adsorbed or desorbed, while the solution concentration, which under the experimental conditions is very close to the interfacial bulk concentration, may be varied slowly in a controlled way.

The glass tubing connected to the outlet of the reaction cell is placed in the well of the γ detector and permits continuous monitoring of the bulk concentration against time. When proteins are instantaneously added to the solution, the amount fixed by hydroxyapatite powder after a given time is calculated following settling of the powder and withdrawal of 100–300 μl of solution.

RESULTS AND DISCUSSION

Numerous studies dealing with protein adsorption onto solid surfaces have been published during the last decade. Usually, the solution is brought into contact with the surface for a few hours, say 2–8 hr. It is commonly assumed that (*i*) after this time period, the solution is in equilibrium with the surface and (*ii*) such systems follow a Langmuir adsorption isotherm characterized by a linear dependence of c_s^{-1} on c_b^{-1}, where c_s and c_b are, respectively, the "equilibrium" surface concentration and bulk protein concentration. A classic Langmuir isotherm obtained in our apparatus is shown in Fig. 1. In these experiments, an albumin solution was instantaneously added to the cell containing hydroxyapatite powder and adsorption was allowed to take place for about 8 hr. Stirring was then stopped so that the powder could settle. After analysis of the supernatant solution, the amount adsorbed was estimated by applying the mass balance equation

$$Sc_s = V[c_b(0) - c_b], \quad [1]$$

where S represents the surface area of the sample, V is the volume, $c_b(0)$ the protein concentration initially added, and c_b the concentration at the term of the considered time interval. Observing the linearity of the plot c_s^{-1} versus c_b^{-1} (Fig. 2), it may be concluded that, as for other systems, the adsorption of albumin onto hydroxyapatite is well described by the Langmuir model. However, it must be pointed out that such a representation is particularly sensitive to low bulk concentrations, whereas the higher concentrations are located in a very restricted domain of the figure. Since the Langmuir model does not accurately take into account interactions between adsorbed particles (hard-sphere interactions, coulombic interactions, etc.), it may be expected to be best adapted for low surface coverage, as easily verified for

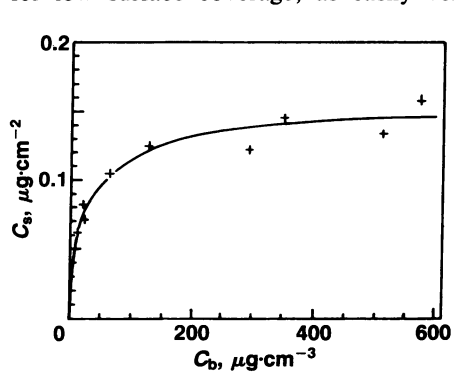

FIG. 1. Adsorption isotherm obtained after 8 hr of equilibration and by immediate addition of entire amount of protein to the bulk solution.

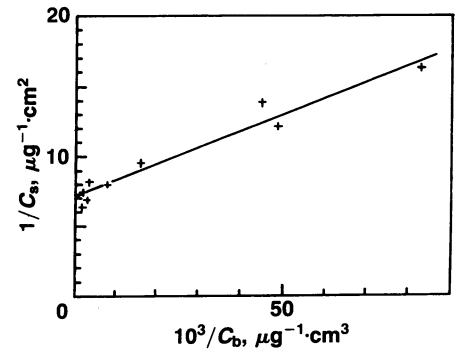

FIG. 2. Plot of $1/c_s$ vs. $1/c_b$ for the experiments described in Fig. 1.

hard-sphere adsorption. If we limit our analysis to this stage, we might claim to have demonstrated the validity of such a model, for the system under study.

The cornerstone of the Langmuir model is that it implies a "dynamic equilibrium" between the surface and the bulk solution. This means that c_s depends only on the bulk concentration c_b and not on the history of the adsorption process. To investigate this particular point, various dilution experiments were carried out. Apatite was brought into contact with an albumin solution at a given concentration and this solution was then diluted by injection of Tris·HCl buffer, at different rates. Injection rates (Q_V) were chosen so that the concentration changes at the outlet of the cell could be followed with good precision as a function of time. The interfacial concentration changes were then deduced from

$$Sc_s(t) = Sc_s'(0) - Q_V \int_0^t c_b(u)du + V[c_b'(0) - c_b(t)], \quad [2]$$

where $c_s'(0)$ and $c_b'(0)$ are, respectively, the interfacial and bulk concentrations at the beginning of the buffer injection step ($t = 0$). Eq. 2 is, again, a simple mass balance equation, valid at any time $t > 0$. In the absence of an adsorbing or desorbing surface, when buffer solution is injected into the cell containing an activity A_0, the activity $A(t)$ at the outlet is given by

$$A(t) = A_0 \exp(-Q_V t/V). \quad [3]$$

Excellent agreement is obtained between theoretical and experimental dilution curves (22), which shows that the dilution process and the counting technique are well and precisely controlled. Experimental curves related to dilution in the presence of the adsorbent are given in Fig. 3. Two main observations are to be noted. (*i*) When the dilution of the bulk solution starts at a point located below the isotherm curve in the (c_s, c_b) space, the curve describing the variation of c_b during the dilution process crosses the isotherm. Even if the crossing point is reached after only 3–4 hr of adsorption (see curve C of Fig. 3), the fact that the amount adsorbed does not decrease when the bulk concentration is later decreased means that the classic isotherm determined after 8 hr of adsorption does not correspond to an equilibrium situation: c_s is a function of the history of the surface. This behavior is typical of irreversible processes. (*ii*) It appears, however, that the amount adsorbed at the plateau, $c_{s,max}$ (0.15 $\mu g \cdot cm^{-2}$) of the classic isotherm can never be exceeded, regardless of the history of the system.

This last point was further checked by means of several experiments in which surface coverages were obtained by fundamentally different experimental pathways. The recorded variations of the bulk concentrations against time are summarized in Fig. 4 and the corresponding amounts of

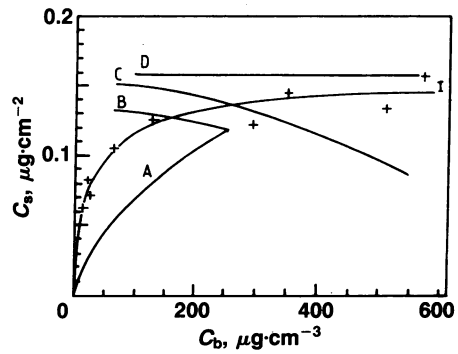

FIG. 3. Adsorption–dilution experiments. Curve I: 8-hr isotherm (given in Fig. 1). Curve A–B: slow adsorption of albumin (c_0 = 66 μg·cm^{-3}) over 8 hr at Q_V = 4.31 × 10^{-4} cm^3·s^{-1} (A), followed by dilution through injection of buffer at the same Q_V value (B). Curve C: adsorption of albumin (c_0 = 695 μg·cm^{-3}) over 30 min, by instantaneous protein addition followed by dilution at Q_V = 4.44 × 10^{-4} cm^3·s^{-1}. Curve D: adsorption of albumin (c_0 = 858 μg·cm^{-3}) over 8 hr, by instantaneous protein addition followed by dilution at Q_V = 4.44 × 10^{-4} cm^3·s^{-1}. For curves C and D, only the dilution stages are given.

albumin adsorbed are given in the figure legend. The differences in c_s from one experiment to another are not significant. Thus, it can be concluded that the maximum amount of human serum albumin that can be fixed by hydroxyapatite is close to 0.15 μg·cm^{-2}, regardless of the history of the adsorption process. A comparable value was found in the bovine serum albumin/poly(dimethylsiloxane) system (23). This is an unexpected result if one compares it to the adsorption data for another elongated molecule, fibrinogen, on silica beads. Both molecules have a similar elongation ratio (e = 1/5), and for this latter system, it has been shown (24) that the amount of fibrinogen adsorbed depends strongly on the bulk concentration path followed during the first hour of adsorption. Thus, the higher the initial mass transfer rate $Q_V c_0$ (where c_0 is the protein concentration of the injected solution), the higher the amount of adsorbed protein. We observe that for an elongated molecule, the probability of being trapped by one end in the interfacial force field (in the so-called "end-on" state) is much higher than for direct adsorption (in the "side-on" state). Consequently, three processes have to be considered: (*a*) adsorption of the proteins to the surface; (*b*) surface denaturation in one or the other state, leading to enhanced binding with the surface and thus a decreasing desorption constant; and (*c*) transition between the two states of adsorption, which is almost total in a sense.

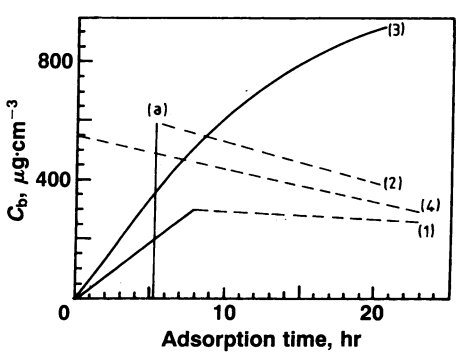

FIG. 4. Variation of the protein concentration in the bulk solution versus time for different experimental conditions. Curve 1: an albumin solution (c_0 = 260 μg·cm^{-3}) was injected into the cell (containing 50.6 mg of hydroxyapatite) at a flow rate of Q_V = 4.44 × 10^{-4} cm^3·s^{-1} for 8 hr (—); stirring without injection was then pursued for 15 hr (---). Curve 2: an albumin solution (c_0 = 97 μg·cm^{-3}) was injected at Q_V = 4.44 × 10^{-4} cm^3·s^{-1} into the cell over 5.5 hr (not visible on this scale). Stirring was then stopped and the hydroxyapatite powder (51.1 mg) was allowed to settle. A more concentrated solution of albumin was added so that the concentration in the bulk became 592 μg·cm^{-3} (a; —). Stirring was then pursued for 15 hr (----). Curve 3: an albumin solution (c_0 = 1000 μg·cm^{-3}) was injected into the cell over 21 hr at Q_V = 4.44 × 10^{-4} cm^3·s^{-1} (—). Curve 4: an albumin solution (c_0 = 550 μg·cm^{-3}) was added directly to the cell. Adsorption was allowed to take place for 24 hr under continuous stirring (----). Amounts of adsorbed albumin at saturation ($c_{s,max}$, μg·cm^{-2}) for the four procedures were as follows: curve 1, 0.149; curve 2, 0.142; curve 3, 0.158; curve 4, 0.151.

In the case of fibrinogen adsorption, time scales associated with these processes have recently been analyzed (P.S., Ph. Déjardin, A. Johner, and A. Schmitt, unpublished data). One observes also for this protein (24) that the maximum surface coverage depends significantly on the thermodynamic path chosen to attain saturation. In principle, such a mechanism could also take place in the albumin/hydroxyapatite system and was indeed observed for albumin/ε-carboxybenzoxylysylleucine (25). However, for the present system, the independence of the highest adsorption with respect to the surface-coverage history means we can assume that most of the adsorbed albumin molecules are in a side-on configuration. If one considers the size of an albumin molecule to be 2.7 × 2.7 × 11.6 nm^3 (26), the maximum coverage of the surface would be, for side-on adsorbed molecules, of the order of 40%. This is a reasonable estimate, which also means that the adsorbed layer corresponds to a monolayer.

From the preceding experiments, it appears that the plateau value of 0.15 μg·cm^{-2} is independent of the history of the adsorption process and that the system is not at equilibrium after 8 hr of adsorption. The question now to be answered is whether, after a prolonged adsorption time, the system evolves to equilibrium and what is then the amount finally adsorbed. An experiment was therefore performed in which the cell containing apatite was initially filled with a protein solution at low concentration (10 μg·cm^{-3}) and a solution of equal concentration was then injected over a period of >100 hr. After 50, 75, or 100 hr of adsorption the system had not yet reached its equilibrium point (Fig. 5). However, it can be estimated that, if one would wait a sufficiently long time, the adsorption would ultimately reach its plateau value corresponding to the real equilibrium point—that is, the adsorption plateau value. It should be remembered that if the adsorption rate were limited only by transport to the surface (as in the Smoluchowski model, where each molecule hitting the surface is adsorbed), the time necessary to attain surface saturation would be, at such a concentration, of the order of a few hours.

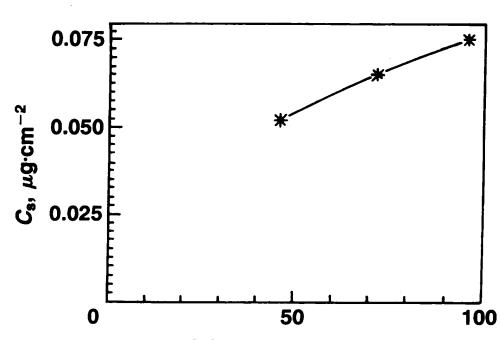

FIG. 5. Amount of protein adsorbed at the hydroxyapatite surface vs. time for a low constant bulk concentration, $c_b(0)$ = 10 μg·cm^{-3}. Experiments were performed by immediate addition of a protein solution at the concentration $c_b(0)$ followed by continuous injection of the same protein solution.

The foregoing analysis is further illustrated by experiments (Fig. 6) in which albumin solutions were added to the cell in order to attain final bulk concentrations of 250–300 $\mu g \cdot cm^{-3}$ after a given lapse of time. As soon as c_s was of the order of 0.15 $\mu g \cdot cm^{-2}$, no further adsorption occurred, because the surface concentration had reached its saturation value. These data show why the plateau value first deduced from the isotherm experiments represented in Figs. 1 and 2 was correct: the system was indeed at equilibrium.

Finally, the adsorption kinetics were followed continuously with time by the following procedure. Albumin at a concentration (c_0) of 0.775 $mg \cdot cm^{-3}$ was injected at a mass transfer rate Q_V of 1.02 $\mu g \cdot s^{-1}$. The amount adsorbed by the surface was calculated with an integral mass balance equation:

$$Sc_s(t) = Q_V c_0 t - Q_V \int_0^t c_b(u)du - c_b(t) \cdot V, \qquad [4]$$

where $c_b(t)$ represents the protein concentration of the solution in the cell at time t estimated by continuous recording of the activity $A(t)$ at the outlet of the cell. The variation of c_s with time was then analyzed with a least-squares fit, using the Langmuir model defined by

$$\frac{dc_s}{dt} = k_a c_b (c_{s,max} - c_s) - k_d c_s. \qquad [5]$$

$c_{s,max}$ was chosen equal to the plateau value (0.150 $\mu g \cdot cm^{-2}$). The quality of the adjustment with the constants k_a and k_d (Table 1) was estimated with the two parameters s^2 (sum of least squares) and σ (relative mean error). It appears that Eq. 5 does not accurately describe the experiment over the entire adsorption domain; however, it fits the experimental data over limited time or concentration intervals. This shows, once again, the nonvalidity of the Langmuir model. Two different physical reasons may explain this behavior. (*i*) The surface exclusion function Φ, which in the Langmuir model is given by $(c_{s,max} - c_s)$, is a more complicated function of the amount adsorbed. Recent theories aimed to provide a rough estimation of this function in the case of hard-sphere adsorption onto solid surfaces (27). Φ must be a decreasing function of the surface coverage. It is equal to 1 for c_s equal to 0 (no adsorbed molecules) and equal to 0 for c_s equal to $c_{s,max}$. If one analyzes such behavior with Eq. 5, it leads to a decrease of k_a and k_d in the higher coverage domains. (*ii*) Another physical process that must be taken into account is superficial protein denaturation. Such a mechanism, suggested by Beissinger and Leonard (7), is irreversible and its direct consequence is that k_d must decrease with time, whereas it has no effect on k_a. Experimentally, one observes a decrease in both k_a and k_d and it is reasonable to assume that both explanations are involved. It is, however, beyond the range of this paper to go further in this analysis on the basis of the present experiments.

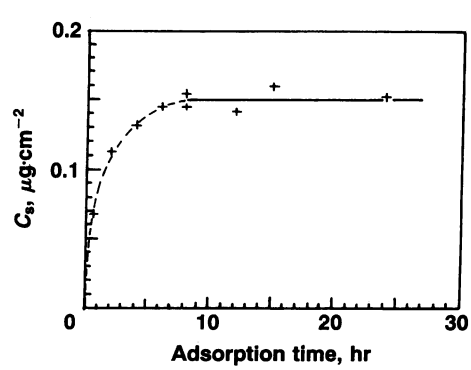

FIG. 6. Amount of albumin adsorbed at the hydroxyapatite surface vs. adsorption time. Experiments were carried out by instantaneous addition of the entire amount of protein to the cell and the solution then remained under stirring without further protein addition. The initial protein concentration was chosen to attain a final bulk concentration of 250–300 $\mu g \cdot cm^{-3}$.

Table 1. Sets of k_a and k_d values used to fit the kinetics given in Fig. 3 according to Eq. 5 by considering several domains

Domain	c_s, $\mu g \cdot cm^2$	$k_a \times 10^{-5}$, $cm^3 \cdot mol^{-1} \cdot s^{-1}$	$k_d \times 10^5$, s^{-1}	$s^2 \times 10^4$	$\sigma \times 10^2$
1	0–0.136	0.081	0.01	2218	6.16
1	0–0.06	1.50	55	0.54	0.8
2	0.06–0.136	0.17	0.14	4.7	0.13
1	0–0.06	1.50	55	0.54	0.8
2	0.06–0.127	0.17	0.087	0.064	0.04
3	0.127–0.136	0.16	0.48	0.09	0.04

The sum of least squares, $s^2 = \Sigma_{i=1}^n [(c_{s\,i,exp} - c_{si,calc})/c_{s,max}]^2$; the relative mean error, $\sigma = \Sigma_{i=1}^n [(c_{si,exp} - c_{si,calc})/c_{s,max}](100/n)$, where $c_{si,exp}$ is the experimentally measured c_s value, $c_{si,calc}$ is the calculated c_s value, $c_{s,max}$ is the amount taken up at saturation, and n is the number of experimental points.

Conclusions. We wished to answer a few questions emerging from an analysis of experimental results that had been published on protein adsorption. The experimental investigations presented here allow us to reach the following conclusions.

(*i*) For the system under study (albumin/hydroxyapatite), the so-called "isotherm" determined from experiments running over a few hours depends on the history of the adsorption process, as long as surface saturation is not attained.

(*ii*) If the adsorption process is pursued for a sufficiently long time, one always attains surface saturation, whatever the bulk concentration. This maximum surface concentration is independent of the adsorption history. In other words, the long time "equilibrium isotherm" degenerates into a simple horizontal curve where $c_{s,max}$ is independent of c_b.

(*iii*) The absolute value of $c_{s,max}$ fits reasonably well with the value predicted for the jamming limit in the model of random sequential adsorption.

(*iv*) It is not clear why it takes so long to approach the plateau value at low bulk concentrations. Two factors may be invoked. The first is related to the adsorption rate, which becomes increasingly small near the jamming limit, this rate being predicted to vary according to a power law $t^{-\alpha}$ ($\alpha \leq 0.5$) (23). The second is due to surface denaturation of the adsorbed molecules. Since the plateau value is independent of the process history, denaturation does not seem to involve surface spreading of the adsorbed albumin molecules. However, optimization of the surface contact increases with time, making desorption less and less probable. Its time dependence remains to be clarified.

We thank Professor A. Schmitt for his constant support and for fruitful discussions on this subject.

1. Horbett, T. A. & Brash, J. L. (1987) in *Proteins at Interfaces: Physicochemical and Biochemical Studies*, eds. Brash, J. L. & Horbett, T. A. (McMaster, Washington) pp. 1–33.
2. Weathersby, P. K., Horbett, T. A. & Hoffmann, A. S. (1977) *J. Bioeng.* **1**, 395–410.
3. Voegel, J. C., de Baillou, N. & Schmitt, A. (1985) *Colloids Surf.* **16**, 289–299.
4. Brash, J. L., Uniyal, S., Pusineri, C. & Schmitt, A. (1983) *J. Colloid Interface Sci.* **95**, 28–36.
5. Brash, J. L. & Samak, Q. (1978) *J. Colloid Interface Sci.* **65**, 495–504.
6. Soderquist, M. E. & Walton, A. G. (1980) *J. Colloid Interface Sci.* **75**, 386–397.

7. Beissinger, R. L. & Leonard, E. F. (1980) *Am. Soc. Artif. Intern. Organs J.* **3,** 160–175.
8. Jennissen, H. P. & Botzet, G. (1979) *Int. J. Biol. Macromol.* **1,** 171–179.
9. Jennissen, H. P. (1981) *J. Chromatogr.* **215,** 73–85.
10. Gorbunoff, M. J. (1984) *Anal. Biochem.* **136,** 425–432.
11. Gorbunoff, M. J. (1984) *Anal. Biochem.* **136,** 433–435.
12. Gorbunoff, M. J. (1984) *Anal. Biochem.* **136,** 440–445.
13. Heimke, J. & Griss, P. (1983) in *Bioceramics of Calcium Phosphate*, ed. de Groot, K. (CRC, Boca Raton), Vol. 4, pp. 80–97.
14. Aptel, J. D., Thomann, J. M., Voegel, J. C., Schmitt, A. & Bres, E. F. (1988) *Colloids Surf.* **32,** 159–171.
15. Thomann, J. M., Mura, M. J., Behr, S., Aptel, J. D., Schmitt, A., Bres, E. F. & Voegel, J. C. (1989) *Colloids & Surfaces* **40,** 293–305.
16. Voegel, J. C., Behr, S., Mura, M. J., Aptel, J. D., Schmitt, A. & Bres, E. F. (1989) *Colloids & Surfaces* **40,** 307–321.
17. Tiselius, A., Hjerten, S. & Levin, O. (1956) *Arch. Biochem. Biophys.* **65,** 132–155.
18. McFarlane, A. S. (1963) *J. Clin. Invest.* **42,** 346–361.
19. Schultze, H. E. & Heremans, J. F. (1966) in *Molecular Biology of Human Proteins with Special Reference to Plasma Proteins—The Plasma Proteins*, eds. Schultze, H. E. & Heremans, J. F. (Elsevier, Amsterdam), pp. 173–235.
20. Aptel, J. D., Voegel, J. C. & Schmitt, A. (1988) *Colloids & Surfaces* **29,** 359–371.
21. Voegel, J. C., Déjardin, Ph., Strasser, C., de Baillou, N. & Schmitt, A. (1987) *Colloids Surf.* **25,** 139–144.
22. Mura-Galleli, M. J. (1990) Ph.D. Thesis, University of Strasbourg, France.
23. Talbot, J., Tarjus, G. & Schaaf, P. (1989) *Phys. Rev. Sect. A* **40,** 4808–4811.
24. Behr, S. (1991) Diplôme de Recherche Spécialisée (Univ. of Strasbourg, France).
25. Soderquist, M. E. & Walton, A. G. (1980) *J. Colloids Interface Sci.* **75,** 386–397.
26. Mizutani, T. & Mizutani, A. (1975) *J. Chromatogr.* **111,** 214–216.
27. Schaaf, P. & Talbot, J. (1989) *J. Chem. Phys.* **91,** 4401–4409.