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Adsorption Kinetics of Protein Mixtures

A Tentative Explanation of the Vroman Effect

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INTRODUCTION

When a surface is exposed to plasma, different proteins from the plasma will adsorb on the surface within several minutes. This adsorption from plasma or serum or protein mixtures depends on the concentration of the various proteins, the nature of the protein and surface, and the adsorption time. Despite numerous studies, the exact composition of the adsorbed protein layer from a multicomponent mixture such as plasma is not yet known.[1-3] Exposure of different surfaces to undiluted plasma results in a relatively limited adsorption of albumin, immunoglobulin, and fibrinogen compared with the adsorption of the same proteins from less concentrated solutions.[4] From experiments of Vroman[5] in which he used normal plasma and plasma lacking high molecular weight kininogen (HMWK), it was concluded that fibrinogen, initially adsorbed on hydrophilic surfaces exposed to normal plasma, is displaced by HMWK. These observations were confirmed by Brash.[3] Observations on the absorption of proteins from various dilutions of plasma showed that no fibrinogen is adsorbed from concentrated plasma, adsorption followed by desorption of fibrinogen occurs for moderately diluted plasma, and a stable adsorbed fibrinogen monolayer is formed for plasma dilutions of about 5% or less.[6-10] The observation that a protein, abundantly present in plasma, predominates initially in the adsorbed protein layer only to be replaced thereafter by a scarce component of the plasma proteins with a higher affinity for the adsorbing surface fits qualitatively into classical binding theory. The dilution effects, however, seem unexplainable in this framework.

In recent studies on the adsorption kinetics of three different proteins on a double layer of phospholipids it was found that the value of the intrinsic adsorption rate constant, k_{on}^{int}, is strongly dependent on the surface concentration of the adsorbed proteins. The experiments showed that k_{on}^{int} decays exponentially with increasing surface concentration Γ. The present study shows that this behavior results in an interaction parameter between the adsorbed proteins that may in principle cause such phenomena as the replacement of fibrinogen by HMWK, and, more importantly, also explains the different types of adsorption from different dilutions of plasma.

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MATERIALS AND METHODS

Materials

A double layer of 1,2-dioleoyl-*sn*-glycero-2-phosphoserine (DOPS) was stacked on a chromium slide as described in refs. 11 and 16. Bovine prothrombin and human fibrinogen were used. The proteins were either obtained commercially or prepared according to established procedures.[16]

Adsorption and desorption experiments were performed by ellipsometry as described in detail previously.[12-14] The surface concentration of proteins was calculated from the measured refractive index and thickness according to the modified Lorenz-Lorentz equation.[15] Sorption experiments were performed in Tris-HCl buffer, pH 7.5, containing 0.1 M NaCl and 1.5 mM $CaCl_2$.

Analysis of Sorption Kinetics

Introduction of Γ-dependent intrinsic adsorption and desorption rate constants, including the deviations from ideal Langmuir adsorption behavior, results in the following set of equations[16,17]

$$\frac{d}{dt}\Gamma(t) = k(\Gamma)^{app}_{on}(\Gamma_{max} - \Gamma(t))c_b - k(\Gamma)^{app}_{off}\Gamma(t) \tag{1a}$$

with

$$k(\Gamma)^{app}_{on} = k(\Gamma)^{int}_{on}D/(D + \delta k(\Gamma)^{int}_{on}(\Gamma_{max} - \Gamma)) \tag{1b}$$

and

$$k(\Gamma)^{app}_{off} = k(\Gamma)^{int}_{off}D/(D + \delta k(\Gamma)^{int}_{on}(\Gamma_{max} - \Gamma)), \tag{1c}$$

where $\Gamma(t)$ = surface concentration ($\mu g/cm^2$) dependent on time t
 $k(\Gamma)_{on}$ = adsorption rate function ($cm^3/\mu g \cdot s$)
 $k(\Gamma)_{off}$ = desorption rate function (s^{-1})
 c_b = protein concentration in the bulk ($\mu g/cm^3$)
 D = diffusion constant of the protein (cm^2/s)
 δ = thickness of the unstirred layer (cm)

The observed values of $k(\Gamma)^{app}_{on}$ and $k(\Gamma)^{app}_{off}$ can be approximated by exponential relations (see FIGURE 2):

$$k(\Gamma)^{int}_{on} = k_{+1}e^{-\alpha\Gamma}$$

and

$$k(\Gamma)^{int}_{off} = k_{-1}e^{+\beta\Gamma}, \tag{2}$$

where α and β are respectively the interaction constants for the adsorption and desorption of this particular protein.[17]

From the preadsorption experiments (see RESULTS) it was concluded that the total amount of adsorbed protein has to be taken into account in these relations; for instance,

for a mixture of two proteins

$$\Gamma = \Gamma_1 + \Gamma_2.$$

The equilibrium association constant K_a is defined by $K_a = k_{on}^{int}/k_{off}^{int}$. Using equations **1b**, **1c**, and **2**, K_a can be written as:

$$K_a = k_{on}^{int}/k_{off}^{int} = k_{on}^{app}/k_{off}^{app} = Ke^{-\gamma\Gamma},$$

where $\gamma = \alpha + \beta$ and $K = k_+/k_-$ is the association constant in the limit of low surface coverage.

In equilibrium one has $d/dt\, \Gamma(t) = 0$ and it follows from equation **1a** that

$$\Gamma_{eq} = (k_{on}^{app}/k_{off}^{app})(\Gamma_{max} - \Gamma_{eq})c_b = Ke^{-\gamma\Gamma_{eq}}(\Gamma_{max} - \Gamma_{eq})c_b.$$

For two components one then obtains:

$$\Gamma_{eq} = \Gamma_{1eq} + \Gamma_{2eq} = (K_1 e^{-\gamma_1\Gamma_{eq}}c_{1b} + K_2 e^{-\gamma_2\Gamma_{eq}}c_{2b})(\Gamma_{max} - \Gamma_{eq}). \qquad (3)$$

Knowing the parameters K_1, K_2, γ_1, γ_2, c_{1b}, c_{2b}, and Γ_{max}, this relation permits calculation of Γ_{eq}. Knowing Γ_{eq} one may then calculate Γ_{1eq} and Γ_{2eq}.

Instead of equation 3 one may also write:

$$\Gamma_{1eq}/\Gamma_{2eq} = [(K_1 c_{1b})/(K_2 c_{2b})]e^{-(\gamma_1 - \gamma_2)\Gamma_{eq}}. \qquad (4)$$

This equation shows that the ratio of the adsorbed proteins is dependent on Γ_{eq}. If Γ_{eq} is
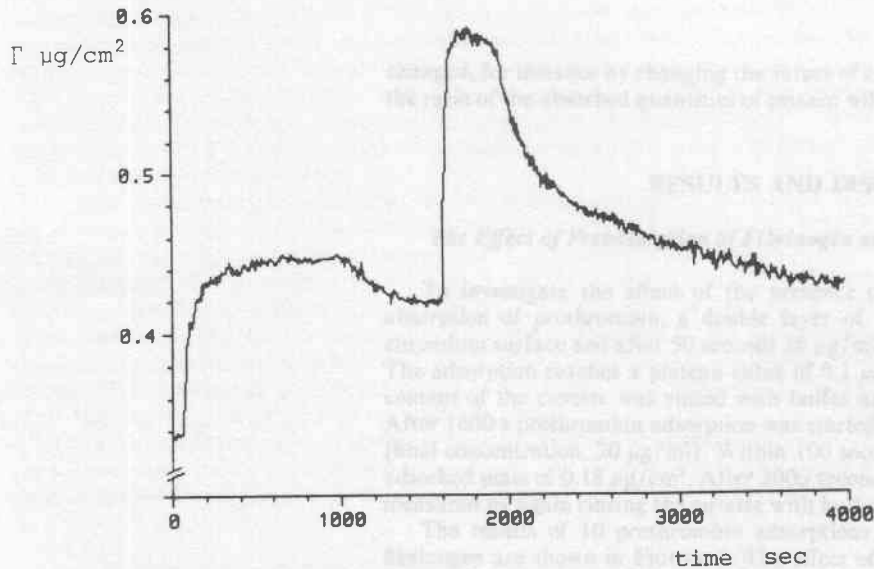

FIGURE 1. Measurement of adsorption and desorption of prothrombin, added 1600 s after preadsorption of fibrinogen (see text). The initial value of $\Gamma = 0.335\ \mu g/cm^2$ (at $t = 0$) was calculated for the DOPS double layer.
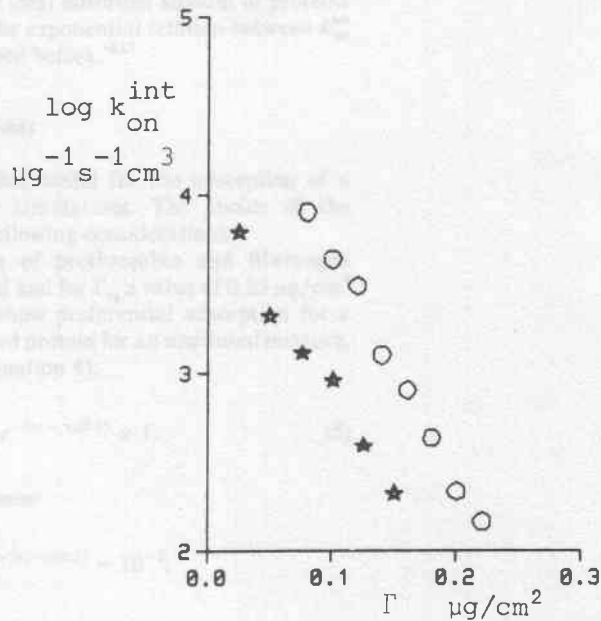
FIGURE 2. The effect of surface concentration Γ on the value of k_{on}^{int} (logarithmic plot); $O =$ without preadsorption of fibrinogen; $*$ = with preadsorption of 0.08 $\mu g/cm^2$ fibrinogen.

changed, for instance by changing the values of c_{1b} and c_{2b} by the same dilution factor, the ratio of the absorbed quantities of protein will also change.

RESULTS AND DISCUSSION

The Effect of Preadsorption of Fibrinogen on the Adsorption of Prothrombin

To investigate the effect of the presence of another absorbed protein on the absorption of prothrombin, a double layer of DOPS was stacked on a reflecting chromium surface and after 50 seconds 20 $\mu g/ml$ fibrinogen was added to the cuvette. The adsorption reaches a plateau value of 0.1 $\mu g/cm^2$ after 1000 s (FIGURE 1). The content of the cuvette was rinsed with buffer and a small desorption was observed. After 1600 s prothrombin adsorption was started by adding the protein to the cuvette (final concentration, 20 $\mu g/ml$). Within 100 seconds adsorption was completed at an adsorbed mass of 0.18 $\mu g/cm^2$. After 2000 seconds the desorption of prothrombin was measured by again rinsing the cuvette with buffer.

The results of 10 prothrombin adsorptions with and without preadsorption of fibrinogen are shown in FIGURE 2. The effect of a preadsorption of fibrinogen (0.08 $\mu g/cm^2$) on the adsorption of prothrombin is to lower the value of k_{on}^{int} by about a factor of ten and to cause a parallel shift to the left of about 0.06 $\mu g/cm^2$. Comparing these values, it is concluded that the adsorbing prothrombin molecules, in first approximation, do not discriminate between preadsorbed fibrinogen or prothrombin. Apparently

the adsorption of one protein can be strongly influenced by the presence of another protein and in the case of a mixture of proteins the total adsorbed amount of proteins has to be taken into account. FIGURE 2 also shows the exponential relation between k_{on}^{int} and the surface concentration Γ, as has been observed before.[16,17]

Computer Simulations

In order to investigate the consequences of this model for the adsorption of a protein mixture, we performed some computer simulations. The choice of the parameter values of the model was guided by the following considerations.

From previous experiments with adsorptions of prothrombin and fibrinogen mixtures a value of $\Gamma_{max} = 0.3$ $\mu g/cm^2$ was estimated and for Γ_{eq} a value of 0.25 $\mu g/cm^2$ was chosen arbitrarily. If the first protein must show preferential adsorption for a highly diluted mixture and be displaced by the second protein for an undiluted mixture, the following requirements must be satisfied (cf. equation 4):

$$K_1 c_{b1} \gg K_2 c_{b2} \simeq 1 \quad \text{and} \quad \frac{K_1 c_{b1}}{K_2 c_{b2}} e^{-(\gamma_1 - \gamma_2)0.25} \ll 1. \tag{5}$$

Therefore, the following numerical values were chosen:

$$K_1 c_{b1} = 10^4; \, K_2 c_{b2} = 10; \quad \frac{K_1 c_{b1}}{K_2 c_{b2}} e^{-(\gamma_1 - \gamma_2)0.25} = 10^{-2},$$

resulting in $\gamma_1 - \gamma_2 = 45$ $(cm^2/\mu g)$.

For the dynamic simulations, values of $k_{+1} = k_{+2} = 1$ $cm^3/\mu g \cdot s$ were chosen. For proteins with molecular weights of 100,000, this value corresponds to an association rate constant of approximately 10^8 M^{-1}/s. Thus a high, and identical, value of k_1 was chosen for both proteins, about one order of magnitude lower than the upper limit as predicted by Smoluchowsky theory. For the bulk concentrations protein values of $c_{b1} = 1000$ $\mu g/cm^3$ and $c_{b2} = 10$ $\mu g/cm^2$ were chosen, that is of the order of the plasma concentrations of fibrinogen and prothrombin, and the corresponding values of k_- thus became: $k_{-1} = 0.1$ s^{-1} and $k_{-2} = 1$ s^{-1}. A value of $D/\delta = 10^{-3}$ cm/s was chosen in Equation 1, in agreement with ref. 16.

It should be stressed, however, that the simulation results shown in FIGURES 3–5 are determined qualitatively by the conditions in equation 5 and do not depend critically on the other assumed parameter values. Also, the picture remains qualitatively unaltered if it is assumed that the effects of the absorbed quantities of both species are not strictly additive. This is suggested, for instance, by the finding that preadsorption of fibrinogen is slightly less effective in lowering k_{on} for prothrombin than preadsorption of prothrombin itself. Such effects could be expected for proteins having different values of γ and could be simply accounted for by assuming a relation of the type $\Gamma_{eq} = \Gamma_{1eq} + f\Gamma_{2eq}$, with f as a weight factor.

The above estimated value of γ, the interaction constant, of about 45 $cm^2/\mu g$ is in good agreement with the experimental values found. For the system tested until now γ values between 20 $cm^2/\mu g$ and 80 $cm^2/\mu g$ were found.[17]

The Ratio of the Adsorbed Proteins as a Function of Time and Dilution

FIGURE 3 shows the sequence and ratio of the adsorption of the two proteins. Initially the protein with the highest $K c_b$, value is adsorbed. At a surface concentration

of about 0.14 μg/cm² the cooperative interaction term strongly influences $\Gamma_{1,\infty}/\Gamma_{2,\infty}$ so strongly that the protein with the largest interaction constant (the second protein γ) can replace the first protein.

The concentrations of the adsorbed proteins as a function of dilution are presented in FIGURE 4. It is shown that, although the total of protein concentration in the solution does not change upon dilution there is a change in the ratio of absorbed proteins. The effect of different values of the interaction constants is shown in FIGURE 5 for a coupled influence, $\gamma = 15$–33 curves.

The significance of an "interaction constant" between protein molecules needs some comment, as has been pointed out recently. In a certain sense it describes the situation where some real chemical or physical attraction or repulsion, force
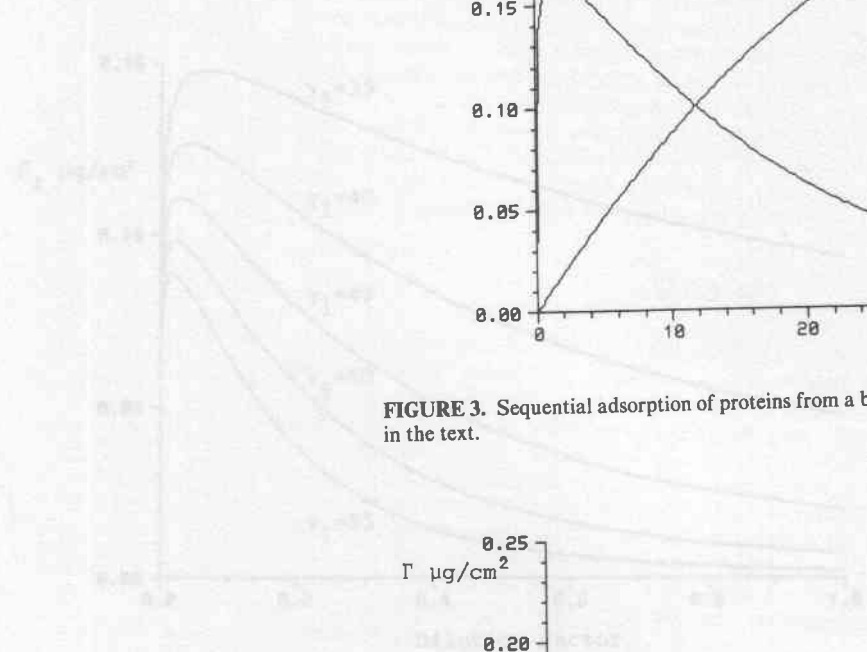

FIGURE 3. Sequential adsorption of proteins from a binary mixture. Parameter values are given in the text.
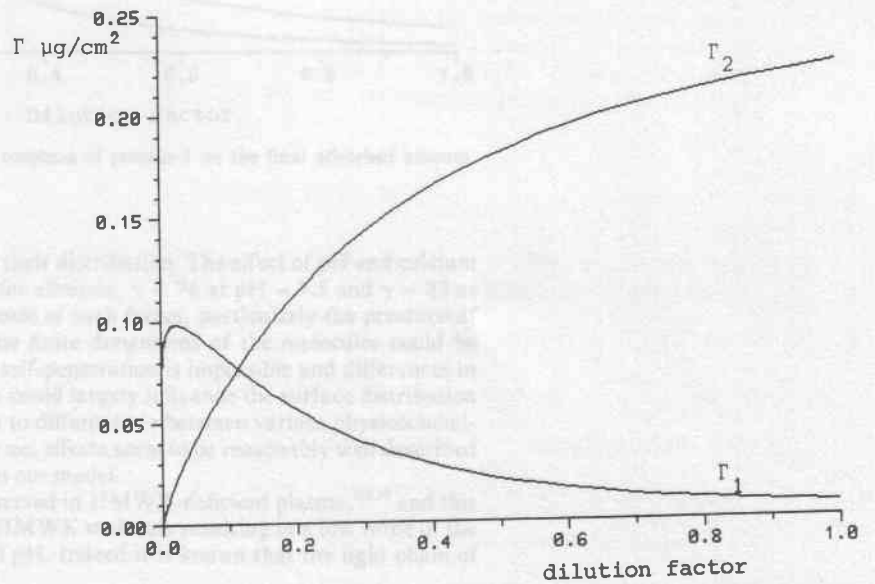

FIGURE 4. The effect of dilution on the final surface concentrations of the proteins from a binary mixture. Parameter values are as in FIGURE 3.

of about 0.16 $\mu g/cm^2$ the exponential interaction term already influences $\Gamma_{1eq}/\Gamma_{2eq}$ so strongly that the protein with the lower $K c_b$ value and a lower interaction constant γ can replace the first protein.

The concentrations of the adsorbed proteins as a function of dilution are presented in FIGURE 4. It is shown that, although the ratio of protein concentrations in the solution does not change upon dilution, there is a drastic change in the ratio of absorbed proteins. The effect of different values of the interaction constants is shown in FIGURE 5 for a range of values $\gamma_1 = 35-55$ cm$^2/\mu$g.

The significance of an "interaction constant" between protein molecules needs some comment, as has been pointed out recently.[18] In a narrow sense it describes the situation where some real chemical or physical, either attractive or repulsive, forces
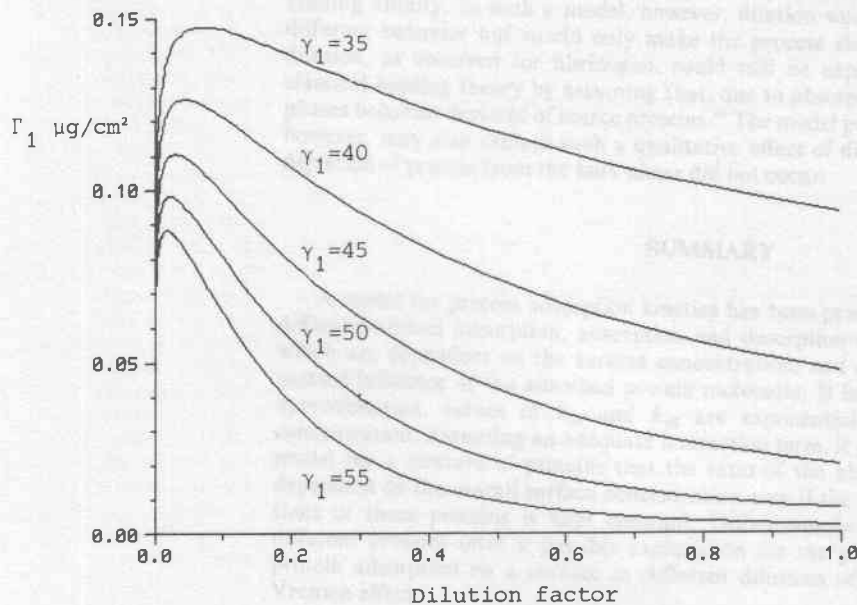

FIGURE 5. The effect of the interaction constant of protein 1 on the final adsorbed amount. Other parameter values are as in FIGURE 3.

exist between molecules that influence their distribution. The effect of pH and calcium ions on the interaction constant (e.g., for albumin, $\gamma = 76$ at pH $= 7.5$ and $\gamma = 23$ at pH $= 6.0$) strongly suggests the existence of such forces, particularly the presence of electrostatic interactions. However, the finite dimensions of the molecules could be sufficient to explain such effect. Their self-penetration is impossible and differences in molecular dimensions and orientations could largely influence the surface distribution of different proteins. It will be difficult to differentiate between various physicochemical forces and spacing effects, but their net effects seem to be reasonably well described by the exponential constants α and β in our model.

Fibrinogen displacement is not observed in HMWK-deficient plasma,[5,9,10] and this suggests some special property of the HMWK molecule resulting in a low value of the interaction constant γ at physiological pH. Indeed it is known that the light chain of

HMWK is responsible for the binding to negatively charged surfaces,[19] and this light chain has a rather unique amino acid composition with an average isoelectric point at pH 7.4. This implies that a large part of the molecule is uncharged at physiological pH, which could favor a low value of γ. It is tempting to speculate about such relations between the values of γ for different proteins and their amino acid composition and function. The same is true for a possible regulatory role of changes in the value of γ as a result of local triggers such as changes in pH or calcium concentrations. Such changes could cause altered compositions of a mixture of adsorbed proteins.

Sequential adsorption of proteins can be explained in a classical binding model by simply assuming that an abundant protein is displaced by a scarce protein with higher binding affinity. In such a model, however, dilution would not cause a qualitatively different behavior but would only make the process slower. A qualitative effect of dilution, as observed for fibrinogen, could still be explained in the framework of classical binding theory by assuming that, due to absorption, the highly diluted bulk phases becomes depleted of scarce proteins.[10] The model presented in the present study, however, may also explain such a qualitative effect of dilution in those cases[8] where depletion of protein from the bulk phase did not occur.

SUMMARY

A model for protein adsorption kinetics has been presented. This model includes diffusion-limited adsorption, adsorption and desorption rate constants k_{on} and k_{off}, which are dependent on the surface concentration, and an interaction term for the mutual influence of the adsorbed protein molecules. It has been shown that, in first approximation, values of k_{on} and k_{off} are exponential functions of the surface concentration. Assuming an adequate interaction term, it is possible to show with this model for a mixture of proteins that the ratio of the absorbed proteins is strongly dependent on the overall surface concentration even if the ratio of the bulk concentrations of these proteins is kept constant. Differences in interaction terms for the different proteins offer a possible explanation for the peculiar behavior of plasma protein adsorption on a surface at different dilutions of the plasma, the so-called Vroman effect.

ACKNOWLEDGMENT

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