

# Adsorption kinetics of protein mixtures

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ADSORPTION KINETICS OF PROTEIN MIXTURES. A TENTATIVE EXPLANATION OF "THE VROMAN EFFECT"

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Abstract: A model for protein adsorption kinetics is presented. This model includes diffusion limited adsorption, adsorption and desorption rate constants which are dependent on the surface concentration and an interaction term for the mutual influence of the adsorbed protein molecules. It is shown that, in first approximation, the values of the adsorption and desorption rate constants are exponential functions of the surface concentration. Assuming an adequate interaction term it is possible to show with this model for the adsorption kinetics of a mixture of proteins that the ratio of the adsorbed 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 behaviour of plasma protein adsorption on a surface at different dilutions of the plasma, the so called "Vroman effect".

INTRODUCTION

In recent ellipsometric studies on the adsorption kinetics of three different proteins, albumin, fibrinogen and prothrombin on a double layer of phospholipid, a model for protein adsorption kinetics was developed (1,2,3,4). This model includes diffusion limited adsorption, adsorption and desorption rate constants  $k_{on}$  and  $k_{off}$  which are dependent on the surface concentration, indicating interaction between adsorbed protein molecules. It was shown that, in first approximation, values of  $k_{on}$  and  $k_{off}$  are exponential functions of the surface concentration. Using these results it is presently shown for a mixture of proteins that the ratio of the adsorbed 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 thus offer a possible explanation for such phenomena as the replacement of fibrinogen by HMWK<sup>\*)</sup> and, more importantly, <sup>\*)</sup>HMWK: High Molecular Weight Kininogen

also offer a tentative explanation for the different types of adsorption from different dilutions of plasma, the so called "Vroman effect" (5,6).

#### MODEL OF PROTEIN ADSORPTION

The observed values of  $k(\Gamma)_{\text{on}}^{\text{int}}$  and  $k(\Gamma)_{\text{off}}^{\text{int}}$  are approximated by the following exponential relations

$$k(\Gamma)_{\text{on}}^{\text{int}} = k_+ e^{-\alpha\Gamma} \quad \text{and} \quad k(\Gamma)_{\text{off}}^{\text{int}} = k_- e^{+\beta\Gamma}$$

where  $\alpha$  and  $\beta$  are, respectively, the interaction constants for the adsorption and desorption of this particular protein (3,4). Protein adsorption is then described by

$$\frac{d}{dt}\Gamma = k_+^{\text{app}}(\Gamma)(\Gamma_{\text{max}} - \Gamma) c_b - k_-^{\text{app}}(\Gamma)$$

with

$$\begin{aligned} \Gamma &= \text{surface concentration } (\mu\text{g}/\text{cm}^2) \\ k_+^{\text{app}}(\Gamma) &= k_+ e^{-\alpha\Gamma} D/(D + \delta k_+ e^{-\alpha\Gamma} (\Gamma_{\text{max}} - \Gamma)) \quad (\text{cm}^3/\mu\text{g}/\text{s}) \\ k_-^{\text{app}}(\Gamma) &= k_- e^{+\beta\Gamma} D/(D + \delta k_+ e^{-\alpha\Gamma} (\Gamma_{\text{max}} - \Gamma)) \quad (1/\text{s}) \\ C_b &= \text{protein concentration in the bulk } (\mu\text{g}/\text{cm}^3) \\ D &= \text{diffusion constant of the protein } (\text{cm}^2/\text{s}) \\ \delta &= \text{thickness of the unstirred layer } (\text{cm}) \end{aligned}$$

The adsorption rate functions presented in Fig. 1 are approximately equal to exponential functions (2,3,4).

Preadsorption of  $0.08 \mu\text{g}/\text{cm}^2$  fibrinogen apparently causes a shift in the adsorption rate function of prothrombin that amounts to an approximately equal quantity ( $\mu\text{g}/\text{cm}^2$ ) of prothrombin. Hence, we assume that equation (1) also is valid for the description of the adsorption of a mixture of two proteins if the  $\Gamma$  in the right hand side of (1) is replaced by the total amount of adsorbed protein:  $\Gamma = \Gamma_1 + \Gamma_2$ .

The equilibrium association constant  $K_a$  is defined by  $K_a = k_+^{\text{app}}/k_-^{\text{app}}$  and equals

$$K_a = k_+^{\text{app}}/k_-^{\text{app}} = K e^{-\gamma\Gamma}$$

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

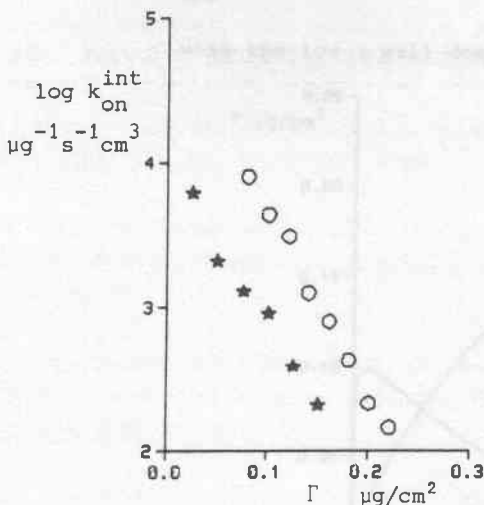


Fig. 1. The effect of the protein surface concentration  $\Gamma$  on the value of the adsorption rate function of prothrombin (20  $\mu\text{g}/\text{ml}$ ) on a double layer of phospholipid (DOPS). 0.05 M Tris-HCl pH=7.5, 0.1 M NaCl and 1.5 mM  $\text{CaCl}_2$ .

O: without preadsorption,

★: with 0.08  $\mu\text{g}/\text{cm}^2$  fibrinogen preadsorbed

For a two component system in equilibrium it is derived that

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

with  $\Gamma_{\text{eq}} = \Gamma_{1\text{eq}} + \Gamma_{2\text{eq}}$ .

This equation shows that the ratio of the adsorbed proteins is dependent on  $\Gamma_{\text{eq}}$ . If  $\Gamma_{\text{eq}}$  is changed, for instance by changing the values of  $c_{1b}$  and  $c_{2b}$  by the same dilution factor, the ratio of the adsorbed quantities of protein will also change.

#### COMPUTER SIMULATIONS

A computer simulation of this model is shown in Fig. 2. It shows that although the ratio of protein concentrations in the solution does not change upon dilution, there is a drastic change in the ratio of adsorbed proteins. The protein with the high  $K$  dominates at low surface coverage, whereas the protein

with the low  $\gamma$  will dominate at high surface concentration.

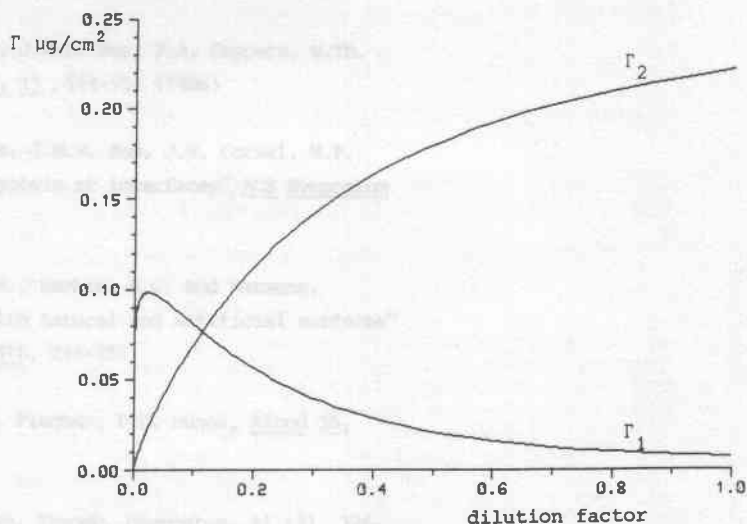


Fig. 2. The concentrations of two adsorbed proteins as a function of dilution.  $K_1 C_{1b} = 10^4$ ,  $K_2 C_{2b} = 10$ ,  $\gamma_1 - \gamma_2 = 45 \text{ cm}^2/\mu\text{g}$ ,  $D/\delta = 10^{-3} \text{ cm/s}$

#### DISCUSSION

Fibrinogen displacement is not observed in HMWK deficient plasma (5,7) and this suggests some special property of the HMWK molecule resulting in a low value of the interaction constant at physiological pH. Indeed, it is known that the light chain of HMWK is responsible for the binding to negatively charged surfaces and this light chain has a rather unique amino acid composition with an average isoelectric point at  $\text{pH}=7.4$ . This implies that a large part of the molecule is uncharged at physiological pH which could favour a low value of  $\gamma$ . It is tempting to speculate about such relations between the values of  $\gamma$  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  $\gamma$  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.

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Note: This model was presented at the New York Academy of Science symposium: Blood in contact with natural and artificial surfaces, nov. 1986 New York.

This model fits well with the interesting kinetic work done by H.P. Jennissen which is also presented in these proceedings.