Experimental studies on the desorption of adsorbed proteins from liquid interfaces

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\textbf{Abstract}

The desorption of proteins from liquid interfaces depends on the conditions under which they have been adsorbed. At low concentrations, the adsorption process takes a comparatively long time and the molecules arriving at the interface have enough space and time to adsorb and unfold. In contrast, adsorption from higher concentrated solutions is faster and adsorbing molecules strongly compete from the beginning of the process. The rate of desorption is studied as a function of the adsorption layer coverage in order to understand to what extend protein adsorption is reversible. The experimental findings cannot give a clear answer on the reversibility, however, the theoretical analysis shows that desorption rates for proteins are many orders of magnitude lower than those for usual surfactants.

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\section{1. Introduction}

Reversibility of adsorption requires, that after the equilibrium is achieved and the protein concentration in the solution is decreased, a new equilibrium with smaller (owing to desorption) amount of protein adsorption should be established. If it does not occur, the adsorption is irreversible. Recently Mac Ritchie (1998) gave a comprehensive discussion of reversibility and irreversibility of protein adsorption at liquid interfaces. He stated that protein adsorption is essentially reversible. For example, the rate of desorption of BSA appears to be dependent on the surface pressure or surface coverage. However, the mechanisms remain for the most part unknown.

Desorption of proteins from liquid interfaces depends essentially on the conditions under which they have been adsorbed. When proteins adsorb at low concentrations, the process takes a comparatively long time and the molecules arriving at the interface have enough space and time to adsorb and unfold. In contrast, adsorption from higher concentrated solutions is much faster and the strong competition of adsorbing molecules starting almost at the beginning of the process hinders unfolding. The rate of desorption is obviously a function of the overall adsorption layer structure.

There are not many experimental techniques suitable for studies of desorption from interfacial layers. The classical way was to adsorb the protein in a Langmuir trough and then exchange the subphase to the pure solvent (Vollhardt, 2003). Drop and bubble shape methods are much more suitable for such experiments as the amount of solution needed is minimum. Svitova et al. proposed a convection cell in which the liquid in a cuvette is exchanged with a known flow rate (Svitova, Wetherbee, & Radke, 2003). The bubble formed in the filled cuvette remains during this exchange and provides information on the subsequent desorption process. Another option was proposed by Wege, Holgado-Terriza, Neumann, and Cabrero-Vilchez (1999) consisting of a special coaxial double capillary. This equipment allows the exchange the volume of a drop,
i.e. to replace the protein solution in the drop completely by the pure buffer solution.

In the present work drop profile analysis experiments are performed to study the desorption process of proteins. The measuring cell was equipped with a special double dosing and coaxial double capillary system suitable to exchange the bulk solution in a drop by the pure solvent without disturbing the continuous analysis of its shape. The experimental data are analysed on the basis of a diffusion controlled adsorption and desorption kinetics model.

2. Experimental

The experiments were performed by a drop profile analysis tensiometer, equipped with a double dosing/coaxial capillary system. The principle of drop profile analysis is to determine the coordinates of a liquid drop from a video image and compare these coordinates with theoretical profiles calculated from the Gauss Laplace equation. This equation contains the surface tension $\gamma$ as the only free parameter. A detailed description of the method is given in Loglio et al. (2001), and of the particular instrument used in Miller, Olak, and Makievski (in press).

The most important detail of the desorption experiment is the coaxial double capillary developed by Wege et al. (1999). The construction is shown schematically in Fig. 1. While the inner capillary is used to control the size of the drop during the experiment, the outer capillary supplies continuously liquid into the drop with a definite flow rate. This flow rate and the period of time for the flow can be selected. When liquid flows into the drop, the video control realises a growing drop and balances the size via the inner capillary. Thus, even during a continuous inflow of liquid the size of the drop can be kept almost constant.

The adsorption/desorption experiments were performed in the following way. First a drop of the respective protein solution is formed and kept constant over a period of about 3 h. Then, a continuous exchange of the drop volume by pure water with a flowrate of 0.2 ml/s is started, i.e. the solution in the drop is exchanged against pure water. The change of surface tension is registered for another 3 h or so. The experiments are performed for $\beta$-CS and $\beta$-LG at different concentrations in pure water.

The samples of $\beta$-lactoglobulin ($\beta$-LG) and $\beta$-casein ($\beta$-CS) from bovine milk were purchased from Sigma Chemical. The proteins were used without further purification. The solutions were prepared with Millipore water.

3. Results

The experimental results of the adsorption/desorption experiments are summarised in Figs. 2 and 3. During the first $10^4$ s, the proteins adsorb at the drop surface. For the higher concentrations, the surface tensions reach a quasi-equilibrium adsorption state, while for the lower concentrations the values just started to decrease. After this initial period, the drop volume is exchanged to pure water in order to remove the protein molecules from the subphase. As one can see, at all concentrations and for both proteins, only a small part of the adsorbed molecules desorbs. Thereafter, the surface tension is changing only slightly: it decreases at the lower and increases at the higher concentrations. While the slight decrease could be understood by a further

![Fig. 1. Schematic of the double capillary system to control a drop and to exchange the liquid in the drop volume.](image)

![Fig. 2. Dynamic surface tension of $\beta$-LG solutions at different concentrations. $c = 5 \times 10^{-8}$ mol/l (•), $c = 1 \times 10^{-7}$ mol/l (○), $c = 2 \times 10^{-7}$ mol/l (■), $c = 5 \times 10^{-7}$ mol/l (▲); continuous bulk exchange with $v = 0.2 \mu$l/s after about $10^4$ s (note the small jump in surface tension after the exchange started).](image)
conformational change of the proteins, the minor increase at higher concentrations points at a further desorption of protein molecules. Note, during the drop bulk exchange with a larger scattering of experimental data points is observed.

The observed behaviour suggests that the protein molecules are essentially irreversibly adsorbed. This would however contradict with the results discussed in a recent monograph (Mac Ritchie, 1998). Therefore, we have to analyse in more detail the adsorption and desorption rates of adsorbing surface active molecules in general, and of proteins as particular case, to draw correct conclusions.

### 3.1. Theoretical analysis of adsorption and desorption rates

When the adsorption rate of a surfactant or a protein is lower than that predicted by the diffusion theory the existence of an adsorption barrier is typically discussed. This seems to be the case for the protein adsorption dynamics, at least during the final stage of the adsorption process (Fainerman, Lucassen-Reynders, & Miller, 1998). In this final stage the adsorption states with the largest molar area mainly disappear (Fainerman, Lucassen-Reynders, & Miller, 2003). Summation of protein adsorptions on all possible states (see equation of the adsorption isotherm (34) in (Fainerman et al., 2003) approximately results to the Frumkin adsorption isotherm equation. Thus, for surfactants or approximately for proteins the adsorption kinetics obeys the equation (Fainerman, 1990):

$$\frac{d\theta}{dt} = \frac{\beta}{\Gamma_{\infty}} \left[ c(0,t)(1-\theta) - \frac{\theta}{b} \exp(-2a\theta) \right]$$

$$= \frac{\beta}{\Gamma_{\infty}} c(0,t)(1-\theta) - a\theta \exp(-2a\theta)$$

(1)

Here $\theta$ is the surface coverage, $\beta$ and $a$ are the adsorption and desorption rate constants, respectively, $b$ is the adsorption equilibrium constant, $a$ is the intermolecular attraction constant, $t$ is the time, $c$ is the concentration, $\Gamma_{\infty}$ is the limiting adsorption. This relationship is also a good approximation for the kinetics of surfactant adsorption when a diffusion mechanism does not hold. It is easy to see, that at the equilibrium (that is, under condition of $d\theta/dt = 0$) Eq. (1) is transformed to the Frumkin isotherm adsorption equation.

From Eq. (1) follows that the constant $\alpha$ can be expressed by

$$\alpha = \beta/b\Gamma_{\infty}.$$  

(2)

Adsorption and desorption involve the processes of overcoming some barriers (either of enthalpy or entropy nature) so that the rate constants can generally be written in the form (Fainerman, 1977):

$$\beta = \beta_0 \exp(-E_a/RT),$$

(3)

$$\alpha = \alpha_0 \exp([-E_a + \Delta G_0]/RT).$$

(4)

$E_a$ and $\Delta G_0$ are the adsorption activation energy and the Gibbs’ adsorption energy, respectively, and the pre-exponential factors $\beta_0$ and $\alpha_0$ correspond to the adsorption and desorption rates for the barrierless adsorption mechanism. In particular, the constant $\alpha_0$ can be regarded to as the rate of the transition between any two states (e.g. those with different conformations) of a molecule in the surface layer. The inverse value $1/\alpha_0$ is the transition time from one state into the other. The value $\Delta G_0$ can be expressed via the adsorption equilibrium constant $\beta$ as (Fainerman, Miller, Aksenenko, & Makievski, 2001):

$$\Delta G_0 = -RT \ln(b\rho)$$

(5)

where $\rho$ is the molar concentration of the solvent. For dilute aqueous solutions $\rho = 56$ mol/l (or $56 \times 10^3$ mol/m$^3$).

Eqs. (4) and (5) with $E_a = 0$ (this case corresponds obviously to the maximum value for the estimate of $\alpha$) yield:

$$\alpha = \alpha_0/b\rho$$

(6)

Therefore, comparing Eqs. (2) and (6), one can express the pre-exponential factor $\alpha_0$ via the parameters $\beta$ and $\Gamma_{\infty}$, i.e. experimental quantities:

$$\alpha_0 = \beta\rho/\Gamma_{\infty}$$

(7)

For example, for $\beta$-casein the respective values are: $\Gamma_{\infty} = 4$ mg/m$^2$ (or $2 \times 10^{-7}$ mol/m$^2$) and $b = 4 \times 10^5$ m$^3$/mol (for the protein molecule as a whole) (Fainerman et al., 2003). Using Eq. (1) and the experimental data given in Miller, Fainerman, Aksenenko, Leser, & Michel (2004), one can show that for $c = 10^{-5}$ mol/l the rate constant is $\beta = 2 \times 10^{-10}$ m/s. Thus, for $\beta$-casein we obtain $\alpha_0 = 56$ s$^{-1}$, i.e. the time during which the protein molecule exists in a certain state is of the order of $1/\alpha_0 = 0.02$ s. This time fits very well into the estimated characteristic time interval of molecular processes (e.g. the denaturation) in protein adsorption layers: $10^{-5}$ s $\leq \tau \leq 1$ s (van Holde, Johnson, & Ho, 1998).

For further estimates we use $\tau = 1/\alpha_0 = 10^{-3}$ s (instead of $1/\alpha_0 = 0.02$ s), according to the values estimated in
van Holde et al., 1998). This value for \( \alpha_0 \) results (for the case of barrierless adsorption, \( E_a \equiv 0 \)) in a desorption rate constant for \( \beta \)-casein of \( \alpha = 4.5 \times 10^{-8} \text{ s}^{-1} \). The value of constant \( \alpha \) will be smaller than specified, if process of \( \beta \)-casein adsorption will go with overcoming of an adsorption barrier, when \( E_a > 0 \). However for simplicity we further approximately assume \( E_a \equiv 0 \), that yields the overestimated value of constant \( \alpha \). For \( \theta \rightarrow 1 \) or \( c(0,t) = 0 \) the first term in the right hand side of Eq. (1) can be neglected and the maximum desorption rate is obtained:

\[
\frac{d\theta}{dt} = \alpha \exp(-2a) \tag{8}
\]

For \( \beta \)-casein with \( a = 1 \) (Fainerman et al., 2003), a maximum relative desorption rate is of the order of \( 10^{-8} \text{ s}^{-1} \) results. From here we can estimate that the time necessary to decrease the surface coverage by 1% is of the order of \( 10^6 \text{ s} \). Note, a value of \( 1/\alpha_0 = 0.001 \text{ s} \) can be an overestimation. Taking \( 1/\alpha_0 = 0.0001 \text{ s} \), we see that during \( 10^6 \text{ s} \) the desorption of the relative \( \beta \)-casein adsorption is only 10%. Obviously, in standard experiments with a duration of less than \( 10^5 \text{ s} \) (about 1 day) the resulting desorption is so slow that it cannot be distinguished from an irreversible behaviour.

For \( \beta \)-LG solutions the constant of adsorption equilibrium \( b \) is smaller than for \( \beta \)-casein (\( 5.3 \times 10^4 \text{ m}^3/\text{mol} \) for the molecule \( \beta \)-LG as a whole). Therefore for expected and close for both proteins values of \( \alpha_0 \), the value of the desorption rate constant \( \alpha \), in the agreement with the Eq. (6), will increase for \( \beta \)-LG approximately in 10 times. This estimation probably is corresponded with the date in Figs. 2 and 3, as for \( \beta \)-LG the desorption is more significant than for \( \beta \)-casein. Let us consider now the situation for a surfactant, in order to see the important difference to adsorbed proteins.

For surfactants the adsorption rate constant \( \beta \) is much larger, i.e. between \( 10^{-8} \) and \( 10^{-6} \text{ m/s} \) (Fainerman, 1985). The highest values correspond to highly surface active non-ionic surfactants. The limiting adsorption \( \Gamma_\infty \) for surfactants is also higher than that of proteins, i.e. between \( 2 \times 10^{-6} \) and \( 5 \times 10^{-6} \text{ mol/m}^2 \). Eq. (7) then yields \( \tau = 1/\alpha_0 = (0.001 \div 0.0001) \text{ s} \), which is a much lower value than the experimental value for proteins (\( 1/\alpha_0 = 0.02 \text{ s} \)). To estimate the constant \( \alpha \) for ionic surfactants, the time necessary for transitions of a molecule between two states could be taken to be \( \tau = 1/\alpha_0 = 10^{-5} \text{ s} \), which approximately corresponds to the condition \( E_a \equiv 0 \). The adsorption equilibrium constant \( b \) is typically in the range between \( 10^{-2} \) and \( 10 \text{ m}^3/\text{mol} \). For example, for sodium dodecyl sulphate (SDS) we have \( b = 0.25 \text{ m}^3/\text{mol} \), while for CTAB \( b = 3.0 \text{ m}^3/\text{mol} \), and for both \( a \equiv 1 \) (Fainerman et al., 2001). Therefore, using Eq. (6) one can calculate \( \alpha = 1 \text{ s}^{-1} \) for SDS and \( \alpha = 0.1 \text{ s}^{-1} \) for CTAB. These values correspond (see Eq. (8)) to very high desorption rate, i.e. 10% desorption within 0.1–1.0 s.

For non-ionic surfactants the constant \( b \) is even higher. For oxyethylated alcohols we get values between \( 10^2 \) and \( 10^4 \text{ m}^3/\text{mol} \) (Fainerman et al., 2001). However, for this case the \( \tau \) values are by 1–2 orders of magnitude lower (i.e. \( \alpha_0 \) values are higher), which leads to desorption constants \( \alpha \) in the range between 0.001 and 1 \text{ s}^{-1} \) (Fainerman, 1985), and for some of these substances we get even as low as \( 0.0001 \text{ s}^{-1} \). Note, in the Frumkin model for oxyethylated surfactants the constant \( a \) is negative (Fainerman et al., 2001). According to Eq. (8) this results in an increase of the desorption rate, thus compensating the effect of the lower \( \alpha \) values. The \( \alpha \) values for these non-ionic surfactants are much higher than those for proteins, but much lower than for ionic surfactants. Therefore, the desorption of highly surface active surfactants can be quite slow, and in some cases a desorption of 10% of the surface layer could need 100–1000 \text{ s} or even more.

4. Summary

The desorption rate for proteins and surfactants is determined mainly by the Gibbs’ adsorption energy, which is related to the adsorption equilibrium constant \( b \). Another factor which affects the desorption rate is the rate of the transition between any two states of a molecule in the surface layer \( \alpha_0 \). The \( b \) values for proteins is by 2–6 orders of magnitude larger than those for usual surfactants, and the \( \alpha_0 \) value for proteins is about 100 times lower. Therefore, the relative desorption for proteins is \( 10^4 \)–\( 10^8 \) times slower than that for usual surfactants.

This explains why with standard experimental techniques desorption processes for proteins are difficult to observe. An experiment over a period of time of only 3 \text{ h}, i.e. \( 10^4 \text{ s} \), is in no way suitable for a decision on the reversibility or irreversibility of adsorbed protein molecules. The presented findings cannot give a clear answer on the reversibility issue, however, the given qualitative analysis demonstrates the requirements which have to be fulfilled for a reliable answer on the question.

References


