Surface Dilational Modulus or Gibbs' Elasticity of Protein Adsorption Layers

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The theoretical model proposed earlier, which assumes that protein molecules in the surface layer can exist in multiple conformations with different molar area, so far has been applied to the equilibrium isotherms of surface pressure, adsorption, and adsorption layer thickness. Here we use the model for the surface dilational modulus, E, measured during area oscillations at given frequencies. Comparing the experimental dependence of the limiting (high-frequency) elasticity, E_0, on the surface pressure for BSA and β-casein with the theoretical ones calculated for the same set of basic model parameters, we find good agreement between the experiments and the proposed theory. The main factor that affects the dependence of E_0 on the concentration or the surface pressure is the dependence of the mean molar area of protein, ω, on the adsorption, Γ. The theoretical value of E_0 for proteins is lower than that characteristic for molecules with constant area in the adsorption layer by the factor of $(1 + \mathrm{d} \ln \omega/\mathrm{d} \ln \Gamma)$. It is shown that for the proteins the derivative $\mathrm{d} \ln \omega/\mathrm{d} \ln \Gamma$ is negative; for example, for flexible β-casein, the value of this derivative approaches -0.9. This value not only produces very low E_0 values but also correctly describes the maximum and minimum values of E_0 measured at fairly low surface pressures.

1. Introduction

The rheological characteristics of protein adsorption layers exhibit an unusual dependence of the surface dilational modulus $E = \mathrm{d}\gamma/\mathrm{d} \ln A$ (where γ is the surface tension and A is the surface area) on the bulk concentration or on the adsorption of the protein.[1−10] For globular proteins, a monotonic increase of the elasticity with increasing adsorption or surface pressure up to a maximum value is observed, and this maximum is considerably higher than that for flexible ones. In contrast, for flexible proteins, E not only is several times lower at its maximum than for the globular ones but also passes through another maximum, followed by a minimum, at lower values of the surface pressure or the adsorption.[2] Many previous theoretical models have failed to explain this behavior, possibly because these models do not account for the known experimental fact that the conformational distribution of protein molecules depends on the monolayer coverage, and hence the average molecular area becomes smaller with increasing adsorption.[11,12] Recently, a theoretical model intended to explain this behavior of protein was proposed.[13] The model[13] reflects the well-known differences between proteins and ordinary surfactants: a sharp increase in the surface pressure with concentration beyond a certain protein adsorption and a significant increase in the adsorption layer thickness with increasing adsorption for flexible proteins. It was shown in ref 13 for several common proteins that a single set of model parameters suffices to reproduce all experimental dependencies: surface pressure, adsorbed amount, and adsorption layer thickness as functions of the concentration. In the present report, it will be shown that the same set of model parameters provides a quite satisfactory agreement between the theoretical and experimental values of the surface dilational modulus. Moreover, the unusual behavior of the surface dilational modulus in protein adsorption layers could be explained from a physical point of view.

2. Theory

The model proposed in ref 13 assumes that protein molecules can exist in a number of states with different molar areas, varying from a maximum value ($\omega_{\max}$) at very low surface coverage to a minimum value ($\omega_{\min}$) at high surface coverage. Assuming next that the molar areas of two "neighbouring" conformations differ from each other by the value ω_0 (the molar area increment, chosen equal to the molar area of the solvent or the area occupied by one segment of the protein molecule) and that the total number of possible states of the protein molecule is n, one obtains the molar area in the ith state, $\omega_i = \omega_1 + (i - 1)\omega_0$, and the maximum area, $\omega_{\max} = \omega_1 + (n - 1)\omega_0$, where $1 \le i \le n$ and $\omega_1 = \omega_{\min} \gg \omega_0$.

The equation of state based on a first-order model for both the nonideal entropy and the heat of mixing for the surface layer was derived in ref 13:

$$-\frac{\Pi\omega_0}{RT} = \ln(1 - \theta) + \theta(1 - \omega_0/\omega) + a\theta^2 \qquad (1)$$

where Π is the surface pressure, R is the gas law constant, T is the temperature, ω is the average molar area of the protein, a is a Frumkin-type intermolecular interaction parameter, Γ_i is the protein adsorption in the i^{th} state, $\theta = \omega\Gamma = \sum_{i=1}^{n}\omega_i\Gamma_i$ is the total surface coverage by protein, and $\Gamma = \sum_{i=1}^{n}\Gamma_i$ is the total adsorption of protein.

The equations for the adsorption isotherm for each state (j) of the adsorbed protein are

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$$b_j c = \frac{\omega \Gamma_j}{(1-\theta)^{\omega_j/\omega}} \exp[-2a(\omega_j/\omega)\theta] \quad (2)$$

where c is the concentration of the protein in the solution bulk and b_j is the adsorption equilibrium constant for the protein at the jth state. It is assumed that the values of the b_j constants for all states j from $i = 1$ to $i = n$ are equal to each other, and therefore the adsorption constant for the protein molecule as a whole is $\sum b_j = nb_j$.[13] This assumption and eq 2 serve to calculate the distribution function of adsorptions over various states of the protein molecule:

$$\Gamma_j = \Gamma \frac{(1-\theta)^{(\omega_j-\omega_1)/\omega} \exp\left[2a\theta\dfrac{\omega_j-\omega_1}{\omega}\right]}{\sum_{i=1}^{n}(1-\theta)^{(\omega_i-\omega_1)/\omega} \exp\left[2a\theta\dfrac{\omega_i-\omega_1}{\omega}\right]} \quad (3)$$

Note that in the range of high bulk protein concentrations the equation of state and adsorption isotherm, eqs 1 and 2, should be additionally modified to account for protein aggregation in the surface layer and become dependent on an extra parameter, ϵ.[13] In this concentration range, the dependence of surface pressure on the bulk concentration is quite weak, and in the present publication, this range is disregarded. Equations 1−3 were derived assuming that the protein molar area increment (which could be possibly thought of as the area of the amino acid residue) is approximately equal to the molar area of the solvent, ω_0. The introduction of an additional proportionality factor (greater or lower than 1) between ω_0 and the protein area increment only slightly affects the calculations results.

It is a general feature of the equation-of-state theory leading to eq 1 that a higher total surface coverage by a mixture of unequal-area molecules favors adsorption of molecules requiring a smaller area at the expense of those with a larger area. At high total adsorption, larger molecules are progressively displaced by smaller ones, as demonstrated in the simple case of constant-area molecules.[14] This effect has been described as an illustration of the principle of Braun−Le Châtelier.[11,12] In the present less simple case, this is reflected by the exponential factor $(1-\theta)^{(\omega_j-\omega_1)/\omega}$ in eq 3. This factor implies that the probability of the existence of a protein molecule in the states characterized by larger area becomes essentially lower with the increase of the monolayer coverage θ because $(\omega_j - \omega_1)/\omega > 0$. Therefore, at $\theta \rightarrow 0$, all states are equally probable, and $\omega = (\omega_{min} + \omega_{max})/2$, but with the increase of θ, the ω value becomes smaller and approaches ω_{min} at $\theta \rightarrow 1$. Hence, from eq 3, we obtain $d\omega/d\Gamma < 0$.

This dependence of ω on θ (or Γ) drastically affects the limiting Gibbs' elasticity, E_0, of the protein monolayer, defined as the value of E measured at a frequency where no relaxation processes affect the surface tension in the time scale of the area oscillations. In this limiting case, the value of $E = E_0$ depends only on the equilibrium equation of state. Differentiation of the equation of state (eq 1) with respect to ln Γ (assuming $\omega_0 \ll \omega$, which holds for proteins) and taking into account the ω dependence on Γ results in

$$E_0 = \frac{d\Pi}{d \ln \Gamma} = \frac{RT}{\omega_0}\left[\frac{\theta}{1-\theta} - \theta - 2a\theta^2\right]\left(1 + \frac{d \ln \omega}{d \ln \Gamma}\right) \quad (4)$$

It was shown above that $d \ln \omega/d \ln \Gamma < 0$, and considering the factor in parentheses in the right-hand side of eq 4, it becomes clear that the elasticity modulus E_0 for the adsorption layer of proteins should be essentially lower than that for molecules with constant ω, for which eq 4 has the usual form:[2,10]

$$E_0 = \frac{RT}{\omega_0}\left[\frac{\theta}{1-\theta} - \theta - 2a\theta^2\right] \quad (5)$$

Next we compare the experimental and theoretical dependencies of the surface dilational modulus.

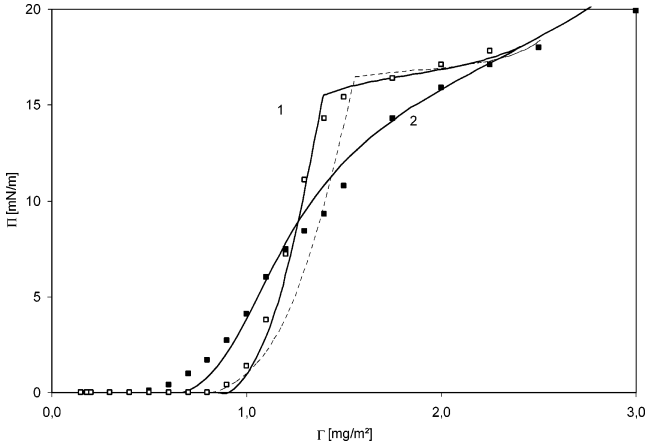

Figure 1. The dependence of the surface pressure for BSA (□, curve 1) and β-casein (■, curve 2) on protein adsorption. Symbols represent experimental data.[2,15,16] Drawn lines represent theoretical curves calculated from eqs 1−3.[13] Dotted line represents theory for a different set of BSA parameters; see text.

3. Results and Discussion

Figure 1 illustrates the experimental and theoretical dependencies of surface pressure on adsorption for BSA and β-casein monolayers. The experimental results shown were reported in refs 2, 15, and 16, and theoretical curves (solid lines) calculated from eqs 1−3 are reproduced from ref 13.

The theoretical curves shown in Figure 1 were calculated for the following values of parameters: for BSA, $\omega_0 = 2.5 \times 10^5$ m^2/mol, $\omega_{min} = 3.0 \times 10^7$ m^2/mol, $\omega_{max} = 7.5 \times 10^7$ m^2/mol, $a = 1$, $b_j = 3 \times 10^5$ L/mol (or $\sum b_j = 5.43 \times 10^7$ L/mol for the molecule as a whole), and $\epsilon = 0.1$; for β-casein, $\omega_0 = 2.5 \times 10^5$ m^2/mol, $\omega_{min} = 4.5 \times 10^6$ m^2/mol, $\omega_{max} = 4.5 \times 10^7$ m^2/mol, $a = 1$, $b_j = 2.5 \times 10^6$ L/mol (or $\sum b_j = 4.075 \times 10^8$ L/mol for the molecule as a whole), and $\epsilon = 0.2$. It is seen that the theoretical curves shown in Figure 1 agree well with the experimental data. It should be noted that the parameter sets above also provide a good description of the surface pressure dependence on the concentration for these proteins.[13]

Comparing the parameters for BSA and β-casein, one can see that the main difference between these proteins is in the values of ω_{min} and ω_{max} and in the relation between these values. For BSA, the ratio $\omega_{max}/\omega_{min} = 2.5$, while for the flexible β-casein, this ratio is much larger: $\omega_{max}/\omega_{min} = 10$. Therefore, the absolute value of the derivative $d \ln \omega/d \ln \Gamma$ for β-casein should exceed that for BSA. The dependence of the derivative $d \ln \omega/d \ln \Gamma$ on the surface pressure for these proteins is shown in Figure 2; it is seen that the values are indeed negative, and for β-casein, the absolute value of this derivative is higher than for BSA: at $\Pi > 15$ mN/m, the derivative $d \ln \omega/d \ln \Gamma$ for β-casein is ca. −0.9.

The theoretical values of the limiting Gibbs' elasticity modulus, E_0, as calculated numerically from eqs 1−3 and from eq 5, are shown in Figure 3. It is seen that the limiting elasticity values calculated from eq 5 (or from eqs 1−3 with $\omega_{max} = \omega_{min}$) show a significant increase of E_0 with increasing surface

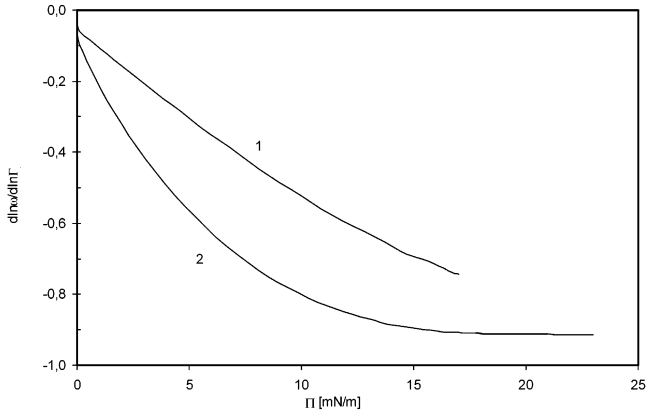

Figure 2. The dependence of the derivative d ln ω/d ln Γ on the surface pressure for BSA (curve 1) and β-casein (curve 2).

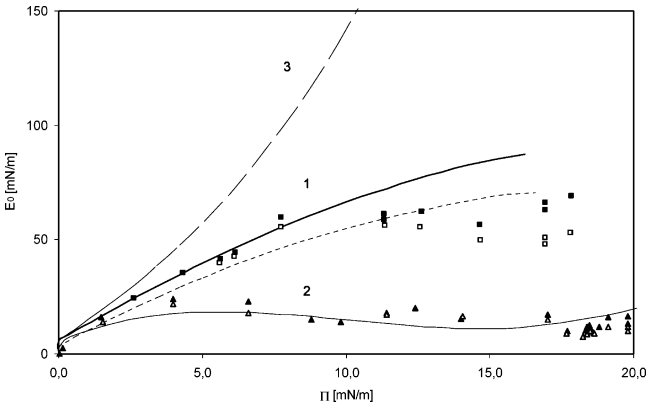

Figure 3. The dependence of the limiting surface elasticity E_0 on the surface pressure calculated from the model eqs 1−4 for BSA (curve 1) and β-casein (curve 2), respectively. Curve 3 was calculated from eq 5. The experimental values of the elasticity modulus E for the BSA at frequencies 0.084 (□) and 0.84 rad/s (■) and for the β-casein at frequencies 0.033 (△) and 0.84 rad/s (▲) are reproduced from refs 2 and 10.

pressure. In contrast, the calculations using the actual ω_{min} and ω_{max} lead to significantly lower E_0 values. For β-casein, not only are these values quite lower than those for BSA, but they also exhibit two extreme values, that is, a maximum followed by a shallow minimum, corresponding to the bulge in the experimental Π vs Γ curve at $\Gamma \approx 1.2$ mg/m^2 in Figure 1. This phenomenon was first noted by Graham and Phillips[3], who qualitatively ascribed it to a transition in the configuration from all-trains to trains-and-loops. The present theory proposes the first quantitative interpretation, as far as we are aware.

Also shown in Figure 3 are the experimental values of the elasticity modulus, E, measured for these proteins using the oscillating drop method.[2,10,17] The data obtained with this method at two values of drop surface oscillation frequency (0.033 and 0.84 rad/s for β-casein and 0.084 and 0.84 rad/s for BSA) are quite similar, which indicates that at these frequencies there is no significant exchange of the protein between the drop surface and the bulk (conservation of the adsorbed mass of protein, $A\Gamma \cong$ const). It follows then that, for the conditions in which these experiments were performed, d ln $A \cong -$d ln Γ and, therefore, $E_0 \cong E$. Moreover, viscous phase angles measured simultaneously[2] were negligible at the highest frequency, implying that the moduli E measured at this frequency were indeed pure elasticities, equal to E_0. Note that the dependence of E_0 on Π as determined in ref 18 for β-casein at a frequency of 0.2 Hz well agrees with the data described in Figure 3. It is seen that the agreement between the theoretical and experimental dependencies presented in Figure 3 is quite good. Not only does the theory correctly predict the limiting elasticity values, E_0, for the two proteins, but it also reproduces in many details the shape of the E_0 vs Π curve: for BSA, this dependence is monotonic and approaches the limiting value, and for β-casein, the curve exhibits extremal behavior with the positions of maximum and minimum of E_0 quite close to those found in the experiments. Using slightly different model parameters for BSA ($a = 0.9$ and $\omega_{min} = 2.7 \times 10^7$ m^2/mol instead of the values $a = 1$ and $\omega_{min} = 3.0 \times 10^7$ m^2/mol) we obtained even better agreement of the theory with experiment for the elasticity modulus (see dotted curve in Figure 3); however, for these values of parameters, the agreement between the experimental and theoretical dependencies of Π on Γ becomes somewhat worse (see dotted curve in Figure 1).

It should be noted that, similar to the protein solutions, also for the solutions of surfactants the assumption that the molar area does not remain constant but becomes lower with the surface pressure increase (which could possibly be ascribed to the variation in the tilt of the hydrocarbon chain with respect to the surface) leads to a better agreement with the experiments. Recent theoretical models,[19−21] which assume internal two-dimensional compressibility of the surfactant molecules in the monolayer, also indicate that some limiting value of E_0 exists (at high concentrations or adsorptions).

4. Conclusions

Measured values of the surface dilational modulus or Gibbs' elasticity can be described satisfactorily by means of a theoretical model[13] that assumes that adsorbed protein molecules can exist in multiple conformations with different molecular areas. The main factor that affects the dependence of the limiting elasticity, E_0, on the concentration or the surface pressure is the dependence of the mean molar area, ω, of protein on the adsorption, Γ. The theoretical value of E_0 for proteins is lower than that characteristic for adsorbed molecules with constant area by the factor of (1 + d ln ω/d ln Γ). This results in lower values of E_0, since configurations with lower molecular areas are favored at the expense of the higher-area configurations, especially at high surface pressures, For the flexible protein, β-casein, the dependence of the average molar area on surface pressure is found to be much stronger than for the globular protein, BSA. This stronger dependence accounts for two characteristic observed differences between the two proteins: (i) surface elasticities are much lower for flexible β-casein than for BSA; (ii) for β-casein, the elasticity E_0 goes through a maximum followed by a minimum at intermediate values of the surface pressure, while in the case of BSA, the elasticity exhibits a monotonic increase with increasing surface pressure.

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