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Kinetics of Adsorption of Globular Proteins at an Air-Water Interface

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Adsorption of globular proteins at an air-water interface from an infinite stagnant medium was modeled as one-dimensional diffusion in a potential field. The interaction potential experienced by an adsorbing molecule consisted of contributions from electrostatic interactions, work done against the surface pressure to clear area at the interface in order to anchor the adsorbed segments, and the change in the free energy due to exposure of penetrated surface hydrophobic functional groups to air. The assumption of irreversible adsorption is employed in the present analysis. The energy barrier to adsorption, present at sufficiently large surface pressures, was found to be higher for smaller surface hydrophobicities, larger surface pressures, larger size molecules, and oblate orientation of an ellipsoidal molecule. Consequently, more adsorption occurred at larger surface hydrophobicities, smaller size molecules, and for prolate orientation of ellipsoidal molecules. The subphase concentration has been shown to be zero at short times, increasing with time at larger times, and eventually becoming close to the bulk concentration as a result of increasing energy barrier to adsorption. The predicted evolution of surface concentration with time for adsorption of lysozyme at an air-water interface agreed well with the experimental data of Graham and Phillips (1979a).

1. Introduction

The strong amphipathic nature of proteins, resulting from their mixture of polar and nonpolar functional groups, causes them to be preferentially adsorbed at gas-liquid as well as liquid-liquid interfaces. This inherent property of proteins enables them to stabilize foams and emulsions. The surface activity of proteins can also be exploited in separation of proteins using foam concentration/fractionation in downstream bioprocessing. Since the diffusion coefficient of proteins is usually an order of magnitude smaller than lower molecular weight surfactants because of their molecular size, rates of adsorption onto air-water and oil-water interfaces are commensurately slower. Consequently, knowledge of the kinetics of adsorption of proteins is necessary for proper understanding of their role in stabilizing foams and emulsions as well as in the design of foam concentration/fractionation column for downstream processing.

Early attempts to model the adsorption of proteins at gas-liquid interfaces assumed that protein adsorption is diffusion controlled. Experimental data (MacRitchie et al., 1963a; Benjamins et al., 1974) on protein adsorption seem to validate such an assumption at high protein concentration. At low concentrations, however, the deviations between the experimental and predicted rates were more pronounced, with the former being much greater than the latter (Benjamins et al., 1974; Damodaran et al., 1988; Graham and Phillips, 1979a). This discrepancy was attributed to the effect of convection currents present in the experiments. Another set of experiments (MacRitchie et al., 1963a) showed that protein adsorption is indeed diffusion controlled at short times, resulting in a steady increase in the surface concentration. At longer times, however, the rate of protein adsorption was found to decrease, thus resulting in leveling off the surface concentration. Such a behavior was attributed to the presence of energy barriers to protein adsorption (MacRitchie, 1978). This energy barrier was shown to be due to surface pressure (MacRitchie et al., 1963b) as well as to an electrical double layer (MacRitchie et al., 1963c). The energy barrier due to surface pressure was manifested in the work a protein molecule arriving at the interface must do in order to clear a sufficiently large area and anchor itself at the interface. On the other hand, the electrical energy barrier is due to the work required to overcome the electrostatic repulsion as a result of the charged interface. Protein adsorption is believed to occur in three steps. The first step is the diffusion of a protein molecule to a subsurface just below the interface, then the protein molecule is adsorbed after it overcomes various energy barriers, and finally it rearranges at the interface (Muramatsu et al., 1963; Graham and Phillips, 1979c), resulting in partial unfolding of the adsorbed segments. Graham and Phillips (1979a-c) in a series of papers examined the adsorption of model proteins such as caesin, BSA, and lysozyme at air-water as well as oil-water interfaces and conclude that the extent of unfolding of globular proteins depends on the surface pressure and that the time scale of the surface rearrangement is much larger than that of adsorption. The surface rearrangement of adsorbed segments of the protein molecule manifests itself in slow change in the surface pressure (Muramatsu et al., 1963; Yamashita et al., 1968). Investigation of adsorption rates of BSA with different degrees of denaturation by Damodaran and Kim (1988) indicated that the denatured protein (which is larger in size) adsorbed faster than a native protein, thus indicating a possible connection between the structure of a protein molecule and the energy barrier to adsorption. At low surface concentrations, the rates of desorption of proteins were found to be negligible (MacRitchie et al., 1963). This is because of the fact that a large number of protein segments are adsorbed at low surface concentrations and that the probability of desorption of all the adsorbed segments is extremely low. Gonzales et al. (1970) measured the rate of desorption of BSA from an air-water interface and found that the energy barrier to desorption

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is very high. MacRitchie (1985) measured the rates of desorption by decreasing the area at a reference surface pressure and found that the rate of desorption of β-lactoglobulin from mixed monolayers is the same as that from a monolayer of β-lactoglobulin but deviated at large times. Hunter et al. (1990) observed that there is no exchange of lysozyme molecules between the air–liquid interface and bulk. On the other hand, adsorbed β-casein was found to exhibit a strong tendency to exchange with bulk (Hunter et al., 1991).

Extensive investigation of diffusion-controlled adsorption of surfactants has been carried out. Ward and Torai (1946) and Sutherland (1952) were among the first to investigate theoretically adsorption kinetics onto a planar fluid–air interface from a quiescent fluid of an infinite extent. Analytical solutions were derived for linear isotherms, and general relationships between surface and subsurface concentrations were developed. This has subsequently been improved by Miller (1981), McCoy (1983), and Borwankar and Wasan (1983) and generalized for other geometries by Mysels (1982), Frisch and Mysels (1983), and Mysels and Frisch (1984). Adsorption and desorption kinetics from finite volume have been investigated for various geometries by Adamczyk and Petlicki (1987). These treatments either assumed local equilibrium or described rates of adsorption and desorption between the subphase and the surface through phenomenological rate constants. Kimizuka et al. (1972) described the adsorption kinetics of surfactants through diffusion in a potential field and solved the equations for simple potentials. Ruckenstein et al. (1976a,b) described reversible adsorption of colloidal particles onto solid surfaces in terms of the interaction potential. Guzmann et al. (1986) described a phenomenological model for kinetics of adsorption of proteins onto an air–water interface. They accounted for the formation of multilayers and the energy barriers for adsorption and desorption. The model parameters were determined by fitting the model to experimental data. This model was extended to the adsorption of lysozyme and β-casein by Hunter et al. (1990, 1991). They postulated that the ellipsoidal lysozyme molecule adsorbs at an air–liquid interface with its major axis parallel to the interface at low surface concentrations and with its minor axis parallel to the interface at high surface concentrations.

A model for the kinetics of adsorption of globular proteins at an air–water interface is proposed that accounts for its surface hydrophobicity, the size and shape of the molecule, and the energy barrier to adsorption. The main driving force for adsorption of proteins is the hydrophobic interactions because of the presence of hydrophobic functional groups exposed to the aqueous medium. Energy barriers to protein adsorption have been accounted for through interaction potentials experienced by an adsorbing protein molecule due to work required to penetrate the interface at a given surface pressure along with hydrophobic and electrostatic interactions. Kinetics of adsorption of globular proteins at an air–water interface from a stagnant infinite medium is described as the diffusion of protein molecule in a potential field. Salient features of the model are described in the first section. This section also describes the evaluation of the interaction potential in terms of surface hydrophobicity, surface pressure, and surface potential for globular proteins. The following section discusses the effects of different variables such as bulk concentration, surface hydrophobicity, and size, shape, and orientation of ellipsoidal molecules on the kinetics of adsorption and compares model predictions with the experimental data. Conclusions are presented in the final section.

2. Model for Adsorption of Globular Protein at an Air–Water Interface

Consider the adsorption of globular protein at an air–water interface. The protein molecule is surface active because of the presence of hydrophobic as well as hydrophilic functional groups. In a globular protein molecule, most of the hydrophobic groups are buried inside the folded, tertiary structure and therefore are inaccessible to the aqueous medium. This shielding of the hydrophobic groups from the aqueous medium gives rise to the globular shape of the protein molecule. In addition to the hydrophilic functional groups, the surface of the globular protein also contains some hydrophobic patches (or domains). The local ordering of water molecules in the vicinity of these surface hydrophobic domains results in the lowering of entropy, thus leading to an increase in free energy. The protein molecule adsorbs at the air–liquid interface such that these surface hydrophobic patches or domains are now exposed to air. This exclusion of hydrophobic patches from the aqueous phase leads to an increase in the entropy. The resulting decrease in the free energy is the driving force for adsorption of globular protein at air–liquid interfaces. Once adsorbed, the penetrated segments of the protein molecule tend to unfold, exposing the hydrophilic groups to the aqueous medium. The degree of unfolding depends on various factors such as the surface concentration, the segment–segment interactions, the number of disulfide bonds, and the structure of the protein molecule.

When the protein solution is exposed to a fresh air–water interface, the diffusing molecule to the interface immediately adsorbs. However, as the surface concentration at the interface builds up, the protein molecule arriving from the bulk has to clear sufficient area at the interface in order to anchor the adsorbing segments. Consequently, the adsorbing molecule has to do work against the surface pressure (MacRitchie and Alexander, 1963a,b; Graham and Phillips, 1979a). In addition, if the pH of the medium is different from the isoelectric point of the protein, the protein molecule is charged. Adsorption of protein at the air–water interface imparts charge to the interface, thus setting up an electrical double layer in the vicinity of the interface. Therefore, in addition to the energy barrier due to surface pressure, a charged protein molecule has also to overcome an electrical energy barrier (MacRitchie and Alexander, 1963c) in order to adsorb.

2.1. Interaction Potential. Figure 1 shows a spherical globular protein molecule adsorbed at the air–water interface. As pointed out earlier, a part of the protein molecule penetrates the interface upon adsorption. The lowering of the free energy of the protein molecule because of exclusion of penetrated surface hydrophobic functional groups from the aqueous medium (referred to hereafter as the "hydrophobic interaction energy") can be evaluated if the type and distribution of these surface hydrophobic patches are known. In order to evaluate the exact lowering of free energy to protein molecule as a result of adsorption, it is necessary to know the type, location, and accessibility of the surface amino acid residues. In the absence of such detailed information, it is assumed that the surface hydrophobic groups are distributed uniformly on the surface. Such an assumption cannot account for preferential orientation of an adsorbing protein molecule because of surface hydrophobic patches. If n_s is the number of surface amino acid residues per unit area of the surface

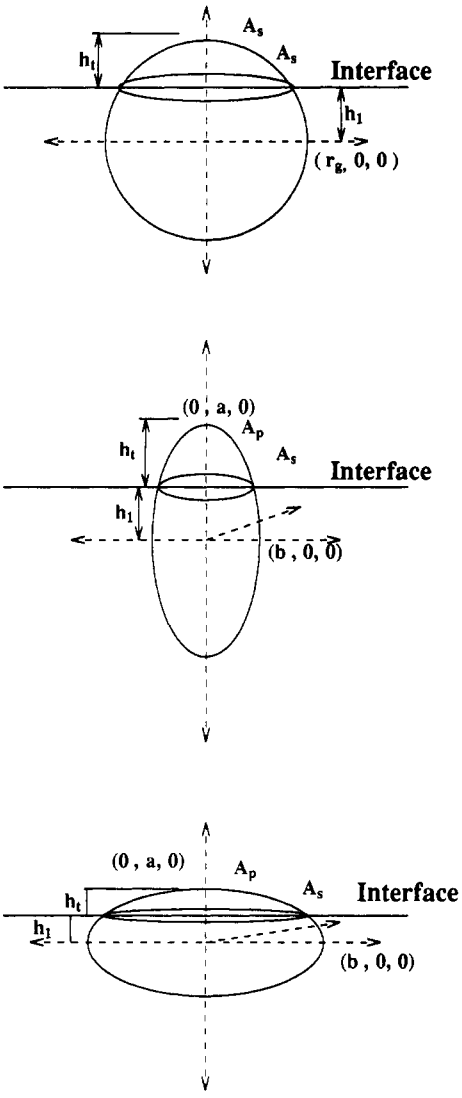

Figure 1. Schematic of spherical, prolate, and oblate ellipsoidal globular protein molecules adsorbed at an air–water interface.

of the protein molecule, A_s is the area of protein molecule that is excluded from the medium (see Figure 1), and h_s is the average surface hydrophobicity per amino acid residue, then the hydrophobic interaction energy ϕ_h is given by

$$\phi_h = -n_s A_s h_s \tag{1}$$

It is to be noted that the surface hydrophobicity arises only from those hydrophobic amino acid residues that lie on the surface and therefore will be smaller than the average hydrophobicity of protein molecule estimated from solubility measurements (Bigelow, 1967). If π_s is the surface pressure, then the work ϕ_s that an adsorbing protein molecule has to do in order to penetrate the interface is given by

$$\phi_s = \pi_s A_r \tag{2}$$

where A_r is the area of the air–water interface (refer to Figure 1) that a protein molecule has to clear. It is to be noted that the surface pressure is a macroscopic property which does not describe detailed microscopic forces between protein segments and the solvent molecules. In writing the above equation, it has been assumed that the globular protein molecule is much larger than the solvent molecule so that a penetrating protein molecule "sees" the average property of the interface. The areas A_r and A_s depend upon the shape and the size of the protein molecules as well as its extent of penetration and can be evaluated from geometric considerations. The expressions for these areas for different shapes can be found in the Appendix.

If q is the net charge on the protein molecule and Ψ is the potential at any location from the air–water interface, then the electrical work ϕ_{el} that needs to be done in bringing the protein molecule from the bulk to that location is given by

$$\phi_{el} = qe\Psi \tag{3}$$

where e is the elementary charge. In general, the charged groups will be distributed along the interface and therefore the potential of the interface due to cationic and anionic groups of the protein molecule can be represented by that of a charge mosaic. The presence of such a mosaic at an air–water interface has been considered by Davies (1951). He has demonstrated the validity of the assumption of uniform electric field as well as the applicability of the Gouy–Chapman equation up to an ionic strength of 0.5. Consequently, from Gouy–Chapman theory, the electrostatic potential Ψ is given by

$$\Psi = 2 \ln\left(\frac{1+\gamma}{1-\gamma}\right) \tag{4}$$

where

$$\gamma = \gamma_0 \exp(-\kappa x) \tag{5}$$

and

$$\gamma_0 = \frac{2kT}{ze} \sinh^{-1}\left[\frac{\Gamma qe}{(8kTn_0\epsilon_0\epsilon_r)^{1/2}}\right] \tag{6}$$

where κ is the Debye–Hückel parameter, x is the distance from the interface, k is the Boltzmann constant, T is the temperature, Γ is the surface number concentration of protein, z is the valence number of electrolyte, n_0 is the number concentration of electrolyte, ϵ_0 is the permittivity of a vacuum, and ϵ_r is the dielectric constant of the aqueous medium.

The net interaction potential ϕ of a globular protein molecule is therefore given by

$$\phi = \phi_h + \phi_s + \phi_{el} \tag{7}$$

It is to be noted that the contribution to the interaction potential from hydrophobic interactions and surface pressure would arise only after the globular protein molecule has penetrated the air–water interface and ϕ_{el} is nonzero only for pH values other than the pI of the protein.

A schematic representation of the interaction potential for adsorption of a globular protein at an air–water interface at the isoelectric point is shown in Figure 2. As the protein molecule penetrates the interface, the interfacial area cleared by the molecule increases with the depth of penetration until half the molecule has penetrated, after which it remains constant. On the other hand, the contribution from the hydrophobic interaction energy increases with the penetration of the molecule as increasingly more hydrophobic groups are exposed to air. The contribution from the surface pressure is predominant at low depths of penetration, whereas the hydrophobic interactions predominate at larger depths of penetration. Consequently, the overall interaction potential increases, reaches a maximum, and subsequently decreases, becoming negative at larger depths of penetration. In other words, an adsorbing protein molecule has to overcome an energy barrier in order to adsorb. Once the energy barrier is overcome, the protein molecule will have a tendency to penetrate the interface as much as possible because of the

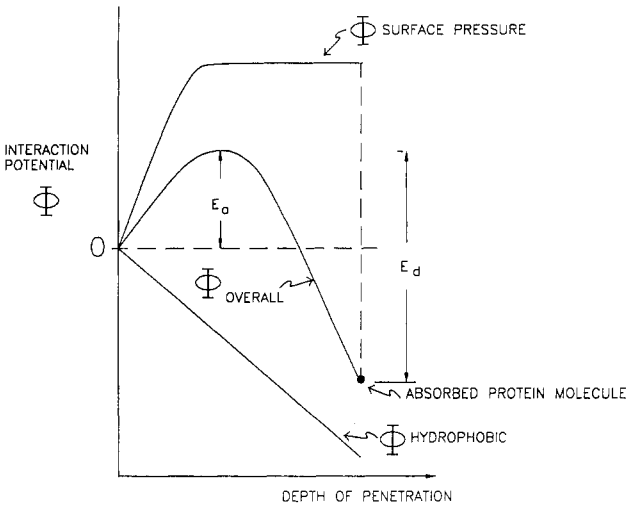

Figure 2. Variation of interaction potential of a protein molecule with the extent of its penetration at the interface. E_a and E_d are the energy barriers for adsorption and desorption, respectively. $\phi_{surface}$ and $\phi_{hydrophobic}$ are the contribution of surface pressure and surface hydrophobicity, respectively, to the interaction potential.

decrease in the interaction potential. As pointed out earlier, the penetrated segments of the protein molecule tend to unfold so as to expose the hydrophilic groups to the aqueous medium. It has been shown by Graham and Phillips (1979) that the time scale of unfolding and rearrangement of adsorbed segments is much larger than the time scale of penetration of globular protein molecules such as bovine serum albumin and lysozyme at an air–water interface. It is, therefore, reasonable to assume that the adsorbing protein molecule more or less maintains its original shape during penetration. Consequently, it is the rate at which the penetrating protein molecule overcomes the energy barrier that influences the rate of adsorption. The area occupied by the adsorbed segments at the interface depends on the extent of unfolding. When adsorbing onto a fresh interface, the protein molecule can penetrate the interface completely since there is no restriction on the amount of area occupied by the molecule at the interface. (The extent of unfolding, may, however, be impeded by the presence of disulfide bonds.) As the surface concentration increases, however, more and more area is excluded because of the steric exclusion of the adsorbed segments belonging to the neighboring molecules. Consequently, the extent of penetration decreases at higher surface concentrations. The steric exclusion of the neighboring segments can be represented by a steep steric barrier as shown in Figure 2. The adsorbed globular protein molecule can, therefore, be considered to be positioned at the potential well. As can be seen from Figure 2, the energy barriers for adsorption and desorption are E_a and E_d, respectively. The energy barrier for desorption is expected to be lower at higher surface concentrations since the extent of penetration of the molecule is smaller.

In order to evaluate the interaction potential of an adsorbing globular protein molecule, the surface pressure as a function of surface concentration should be known. Surface pressure depends upon the surface concentration of adsorbed segments, lateral segment–segment and segment–solvent interactions at the surface, and electrostatic interactions. Unlike the rate of adsorption, therefore, surface pressure is influenced by the extent of unfolding of adsorbed protein molecules. A surface equation of state for globular proteins at an air–water interface has been proposed (Uraizee and Narsimhan, 1991) that accounts for the structure of the protein molecule, its extent of unfolding, and segment–segment, segment–solvent, and electrostatic interactions. This lattice model assumes that all the adsorbed segments are present in the form of trains at the air–water interface. As pointed out earlier, the extent of penetration and unfolding of an adsorbing protein molecule will depend on the surface concentration. This dependence of number of adsorbed segments per protein molecule on the surface concentration is accounted for in the proposed surface equation of state. In this lattice model, the configurational entropy of adsorbed segments is calculated by evaluating (i) the number of ways of placing the adsorbed protein molecules on the surface and (ii) the number of ways of arranging the adsorbed segments of a protein molecule on the surface lattice. The segment–solvent interaction is characterized by the Flory–Huggins χ parameter, whereas the electrostatic interactions are accounted for through the evaluation of the free energy of an electrical double layer set up in the vicinity of the charged interface. The details of the model can be found elsewhere (Uraizee and Narsimhan, 1991). In the present calculations, this model is employed to evaluate the surface pressure of an air–water interface with adsorbed globular protein. (If experimental data of surface pressure vs surface concentration are available, they can be used to evaluate the interaction potential of an adsorbing protein molecule. The advantage of employing the surface equation of state is that calculations can be performed under various conditions at which experimental data of surface pressure may not be available.)

The overall interaction potentials for globular protein molecule at different depths of penetration are evaluated from eqs 1–3 for different surface pressures and surface hydrophobicities. The expressions for the areas A_r and A_s for spherical and ellipsoidal molecules can be found in the Appendix. Figure 3 shows the effect of surface hydrophobicity (top panel) and surface pressure (bottom panel) on the interaction potential of a spherical protein molecule. An increase in the surface hydrophobicity leads to a larger driving force for adsorption and therefore a lower energy barrier. On the other hand, as the surface pressure increases, more work has to be done in order to clear a certain area at the air–water interface. Consequently, the energy barrier for adsorption is higher at larger surface pressures. Similarly, as the size of the spherical protein molecule increases, the area required to anchor the protein molecule at the interface increases, leading thereby to a higher energy barrier to adsorption.

An ellipsoidal protein molecule can adsorb in a large number of orientations and they all lie between the orientations where the major axis is perpendicular to the air–liquid interface (prolate) and where it is parallel to the air–liquid interface (oblate). Hence these two extreme cases are inspected here. Figure 4 shows the interaction potential for prolate and oblate orientations of adsorption. The oblate protein molecule has to do more work to clear the area for adsorption compared to the prolate protein molecule of the same dimension. In addition, more of the surface hydrophobic groups are exposed to the air for the same extent of penetration for a prolate molecule than for an oblate molecule. Thus the energy barrier is higher for an oblate molecule than for a prolate molecule, making the latter a more favorable orientation for adsorption.

Figure 5 shows the effect of an increase in the minor and major axis on the interaction potential of a protein molecule adsorbing in the most favorable state. As the size of the minor axis increases, the area required by a protein molecule to adsorb at the interface increases, and hence the energy barrier to adsorption increases. On the

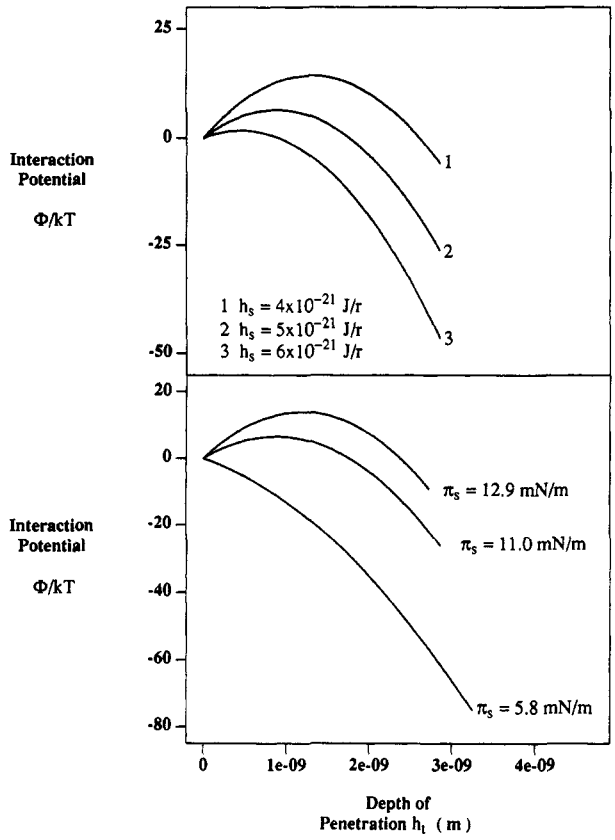

Figure 3. Effect of surface hydrophobicity (top panel) and surface pressure (bottom panel) on the interaction potential for a spherical protein molecule; r_g = 3.0 nm and n_s = 1.88 × 10^{18} residues/m². For the top panel, π_s = 11 mN/m, and for the bottom panel, h_s = 5 × 10^{-21} J/residue.

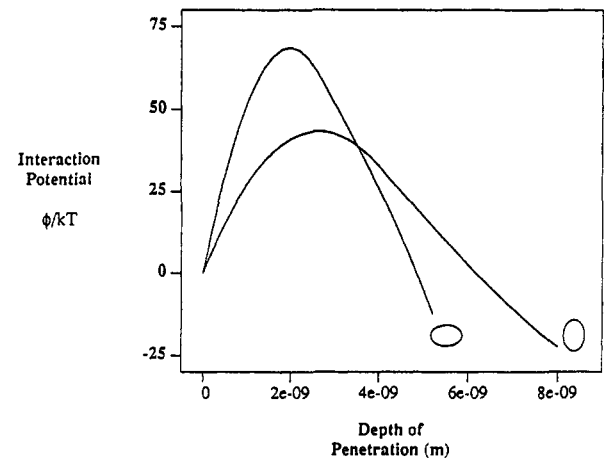

Figure 4. Effect of orientation of an ellipsoidal protein molecule on the interaction potential; protein size 4.0 × 2.6 × 2.6 nm, h_s = 2.5 × 10^{-21} J/residue, n_s = 1.55 × 10^{18} residues/m², and π_s = 9 mN/m.

other hand, as the major axis of the protein molecule increases, the interaction potential decreases as more of the surface hydrophobic groups are exposed to the air.

2.2. Diffusion of Protein Molecule in a Potential Field. The adsorption of a protein molecule from an infinite stagnant medium onto the air–water interface can be considered as one-dimensional un-steady-state diffusion in a potential field and is therefore described by

$$\frac{\partial}{\partial t}c(x,t) = \frac{\partial}{\partial x}\left[D\frac{\partial}{\partial x}c(x,t) + \frac{D}{kT}c(x,t)\frac{d\phi(x)}{dx}\right] \quad (8)$$

where D is the diffusion coefficient, $c(x,t)$ is the protein

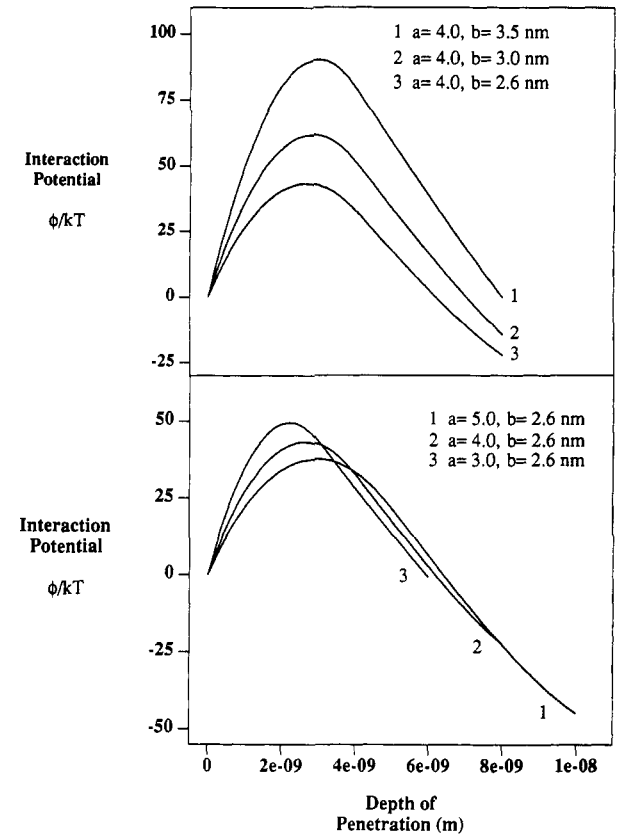

Figure 5. Effect of change of minor axis (top panel) and major axis (bottom panel) on the interaction potential for an ellipsoidal protein molecule adsorbing in its favorable state; h_g = 2.5 × 10^{-21} J/residue, π_s = 9 mN/m, and n_s = 1.55 × 10^{18} residues/m².

concentration at time t at a distance x from the interface, k is the Boltzmann constant, T is the temperature, and $\phi(x)$ is the interaction potential experienced by the protein molecule. If a protein solution of bulk concentration c_b is exposed to a fresh air–liquid interface, the initial concentration profile in the liquid will be uniform. Equation 8 can then be solved with the following initial and boundary conditions

initial condition

$$c(x,0) = c_b \quad \forall x \quad (9)$$

$$\Gamma(0) = 0 \quad (10)$$

boundary condition

$$\lim_{x\to\infty} c(x,t) = c_b \quad (11)$$

$$D\frac{\partial c}{\partial x}(x,t)|_{x=0} = \frac{d\Gamma(t)}{dt} \quad (12)$$

where $\Gamma(t)$ refers to the surface concentration of protein at time t. In addition to the above conditions, one also needs to have information with regard to the subphase concentration of protein near the interface. At sufficiently low concentrations, as pointed out earlier, an adsorbing protein molecule can penetrate the interface more or less completely before unfolding. Therefore, an adsorbed protein molecule is positioned in a deep potential well (refer to Figure 2) because of which the energy barrier for desorption, E_d, is very large. Under such conditions, the

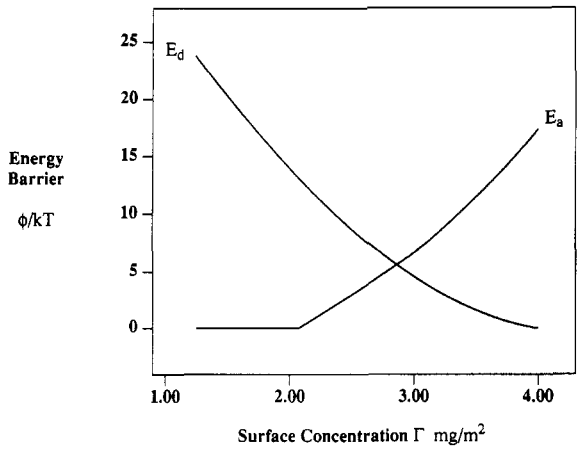

Figure 6. Effect of surface concentration on the energy barrier for adsorption and desorption of lysozyme at an air–water interface.

potential well can be replaced by a sink; i.e., protein adsorption can be considered to be irreversible. Consequently, the subphase concentration is zero, i.e.

$$c(0,t) = 0 \quad \forall t \tag{13}$$

In order to demonstrate the validity of the above condition for sufficiently low surface concentrations, a typical plot of energy barriers for adsorption and desorption of lysozyme versus surface concentration is shown in Figure 6. It is to be noted that the depth of the potential well in which the adsorbed protein molecule is positioned (or, equivalently, the energy barrier for desorption) depends on the extent of penetration of an adsorbing molecule. The variation of this extent of penetration of lysozyme molecule with surface concentration (Uraizee and Narsimhan, 1991) is accounted for in the evaluation of the energy barrier for desorption. The energy barrier for desorption is extremely high at very low surface concentration and decreases at higher surface concentrations. On the other hand, the energy barrier for adsorption increases with surface concentration. Consequently, the energy barriers for adsorption and desorption become comparable only at high surface concentrations comparable to monolayer coverage. This is consistent with the experimental findings of Hunter et al. (1990), who observed that lysozyme, once adsorbed, does not significantly exchange with the bulk, thus implying that adsorption is irreversible. Depending on the structure of proteins, desorption may be important, especially at higher surface concentrations, as demonstrated by Hunter et al. (1991) for casein. When desorption is important, however, the subsurface concentration will be nonzero. The problem of reversible adsorption can be decomposed by the method of superposition and reflection (Mysels, 1982) into two fluxes, one reaching the surface that can be considered as a sink and the other leaving the surface due to desorption. In any event, in the present analysis, the surface is considered to be a perfect sink and the rate of desorption is neglected. Therefore, the model assumes that protein adsorption is irreversible.

Defining the following dimensionless quantities

$$\eta = \frac{c}{c_b} \quad L = \frac{\Gamma_{max}}{c_b} \quad \tau = \frac{tD}{L^2} \quad x' = \frac{x}{L} \quad y = \eta e^{\phi/kT} \quad z = \frac{x'}{(4\tau)^{1/2}}$$

where Γ_{max} is the surface concentration of protein corresponding to monolayer coverage. Equation 8 can be recast in terms of dimensionless variables as

$$\frac{d^2y}{dz^2} - \frac{d\Phi}{dz}\frac{dy}{dz} + 2z\frac{dy}{dz} - 2zy\frac{d\Phi}{dz} = 0 \tag{14}$$

where the dimensionless interaction potential $\Phi = \phi/kT$. The initial and boundary conditions (9), (11), and (13) become

$$z = 0 \quad y = 0 \tag{15}$$

$$z \to \infty \quad y = 1 \tag{16}$$

Since the decay length of the interaction potential is of the order of the radius of a globular protein molecule ($\sim 10^{-8}$–10^{-7} m), it is very much smaller than the length scale of the diffusion boundary layer ($\sim 10^{-5}$–10^{-4} m) except at very short times. The bulk solution can therefore be divided into two regions. Region I is close to the interface where an adsorbing protein molecule will experience the interaction potential and region II is away from the interface where the interaction potential is zero.

Region I. Since region I is much smaller than region II, one can stretch the coordinate in this region by means of the transformation

$$\zeta = z/a \tag{17}$$

where

$$a = R_g/L$$

and R_g is the radius of gyration of the globular protein molecule. Since $\Gamma_{max} \sim 10^{-6}$ kg/m^2, for dilute protein solutions (i.e., $c_b \sim 10^{-4}$–10^{-2} kg/m^3), $L \sim 10^{-4}$–10^{-2} m. Consequently, $a \ll 1$ as $R_g \sim 10^{-8}$ m. In terms of the new stretched coordinate, eq 14 becomes

$$\frac{1}{a^2}\left[\frac{d^2y}{d\zeta^2} - \frac{d\Phi}{d\zeta}\frac{dy}{d\zeta}\right] + \left[2\zeta\frac{dy}{d\zeta} - 2\zeta y\frac{d\Phi}{d\zeta}\right] = 0 \tag{18}$$

Since $a \ll 1$, the first term is much greater than the second. Therefore

$$\frac{d^2y}{d\zeta^2} - \frac{d\Phi}{d\zeta}\frac{dy}{d\zeta} = 0 \tag{19}$$

with the boundary conditions

$$\text{at } \zeta = 0 \quad y = 0 \tag{20}$$

$$\text{at } \zeta = \zeta^* \quad y = \eta^* \tag{21}$$

where ζ^* refers to the interface of regions I and II and η^* is the unknown dimensionless concentration at the interface to be determined by matching the fluxes at the interface of regions I and II. (ζ^* can be chosen such that $\zeta^* \gg$ the decay length of the interaction potential. Since ζ is the stretched coordinate, ζ^* can be taken as ∞ without much error.)

Region II. In the absence of interaction potential eq 14 reduces to

$$\frac{d^2y}{dz^2} + 2z\frac{dy}{dz} = 0 \tag{22}$$

with the boundary conditions

$$z = 0 \quad y = \eta^* \tag{23}$$

$$z \to \infty \quad y = 1 \tag{24}$$

Since the thickness of region I is very much smaller than that of region II, the location of the interface of the two regions can be denoted by $z = 0$ (eq 23). Solving eq 19

with the boundary conditions (20) and (21), solving eq 22 with the boundary conditions (23) and (24), and matching the flux at the interface, one obtains the following equations for the concentration profiles in the two regions and the rate of adsorption:

region I

$$\eta(x,t) = \frac{\eta^*(t)e^{-\Phi}\int_0^{x^*} e^{\Phi}\,dx'}{\int_0^{x^*} e^{\Phi}\,dx'} \tag{25}$$

region II

$$\eta(x,t) = \eta^* + (1-\eta^*)\,\mathrm{erf}\left(\frac{x}{(4Dt)^{1/2}}\right) \tag{26}$$

where the concentration η^* at the interface of the two regions is given by

$$\eta^* = \frac{\int_0^{x^*} e^{\Phi}\,dx}{\int_0^{x^*} e^{\Phi}\,dx + (\pi Dt)^{1/2}} \tag{27}$$

and the rate of adsorption of protein is given by

$$\frac{d\Gamma(t)}{dt} = \left(\frac{D}{\pi t}\right)^{1/2} c_b \frac{(\pi Dt)^{1/2}}{(\pi Dt)^{1/2} + \int_0^{x^*} e^{\Phi}\,dx} \tag{28}$$

If the energy barrier to adsorption is sufficiently high, the contribution to the integral will come only in the vicinity of the maximum. The integral can, therefore, be evaluated by the method of steepest descent to yield

$$\int_0^{x^*} e^{\Phi}\,dx = \int_{-\infty}^{\infty} e^{\Phi_0 - 1/2\Phi''\gamma^2}\,d\gamma = e^{\Phi_0}\left(\frac{2}{\Phi''}\right)^{1/2}\pi^{1/2} \tag{29}$$

where Φ_0 and Φ'' are the dimensionless height of the energy barrier and the second derivative at the maximum, respectively.

The surface concentration $\Gamma(t)$ at time t can then be evaluated from

$$\Gamma(t) = \int_0^t \frac{d\Gamma}{dt}(t')\,dt' \tag{30}$$

The relationship between the surface concentration and surface pressure of a globular protein is required in order to update the interaction potential experienced by the protein molecule during adsorption. The surface equation of state (Uraizee and Narsimhan, 1991) is employed to update the surface pressure during adsorption of lysozyme at an air–liquid interface.

3. Effect of Different Variables on the Kinetics of Adsorption of Globular Proteins at an Air–Water Interface

The evolution of surface concentration of globular protein at an air–water interface with time has been evaluated for different parameters such as bulk concentration, surface hydrophobicity, molecular size, and size and orientation of ellipsoidal molecules. The surface concentration $\Gamma(t)$ was evaluated using eqs 28 and 30. The interaction potential was evaluated from eqs 1–7. The surface pressure π_s at any surface concentration $\Gamma(t)$ was calculated using the proposed surface equation of state (Uraizee and Narsimhan, 1991). Parameter values for lysozyme (Uraizee and Narsimhan, 1991) were employed in all the calculations of surface pressure. The effect of bulk concentration on the kinetics of adsorption of spherical globular protein at an air–water interface is shown in Figure 7. As expected, more adsorption is observed for higher bulk concentrations. As the surface concentration increases, the surface pressure and hence the energy barrier to adsorption increases. This leads to a reduction in the rate of adsorption, eventually resulting in plateauing of the surface concentration. The effect of surface hydrophobicity on the evolution of surface concentration with time is shown in Figure 8. More adsorption takes place for higher surface hydrophobicities. This is understandable since the driving force for adsorption is higher and therefore the energy barrier for adsorption is lower for higher surface hydrophobicities. The effect of protein size on the kinetics of adsorption is shown in Figure 9. Adsorption is slower for larger molecules because of the decrease in the diffusion coefficient. Moreover, the extent of adsorption is more for smaller protein molecules since the energy barrier for adsorption is lower.

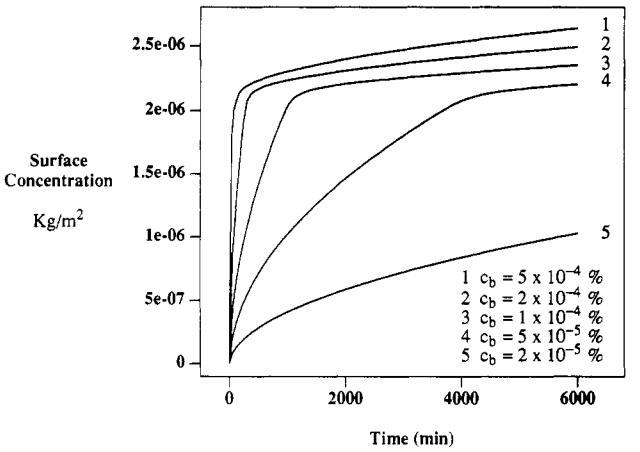

Figure 7. Effect of bulk concentration on the kinetics of adsorption of a spherical protein molecule; $h_s = 6.67 \times 10^{-21}$ J/residue, $n_s = 1.55 \times 10^{18}$ residues/m², and $r_g = 3.0$ nm.

In order to investigate the effect of shape, calculations were done for adsorption of ellipsoidal globular protein molecules. It is to be noted that the rotational Brownian motion of ellipsoidal molecules need not explicitly be accounted for in region II since the diffusion of the molecule is independent of its orientation. In region I, however, the interaction potential experienced by the molecule would depend on its orientation. The rotational Brownian motion of the molecule may not be important once the molecule has penetrated the air–water interface. Consequently, an ellipsoidal protein molecule can adsorb in any orientation. The effect of orientation of an ellipsoidal molecule on the kinetics of adsorption is shown in Figure 10. Two extreme orientations, namely, prolate and oblate, were investigated. At small times, the kinetics of adsorption is independent of orientation since adsorption is diffusion controlled. At large times, however, more adsorption occurs for prolate orientation than oblate orientation. This is because the energy barrier for adsorption of an oblate protein molecule is higher than that for a prolate protein molecule (Figure 4). Of course, the temporal evolution of surface concentration for any other orientation should lie between these two extremes. As pointed out earlier, a protein molecule does not adsorb at a fixed orientation but with the orientation at which it enters region I. Therefore, the actual kinetics of adsorption of an ellipsoidal molecule can be obtained by averaging over all orientations.

Figure 11 shows the effect of change in minor and major axis of a prolate protein molecule on its surface concentration. An increase in the minor axis results in less

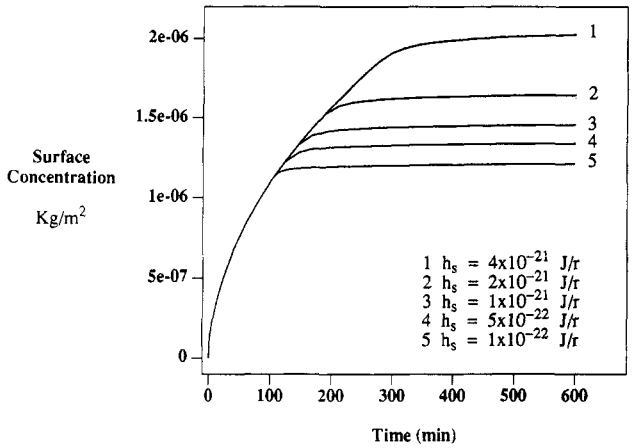

Figure 8. Effect of surface hydrophobicity on the evolution of surface concentration with time; protein size is 7.5 × 3.0 × 3.0 nm, n_s = 1.55 × 10^{18} residues/m^2, and c_b = 2 × 10^{-4} wt %.

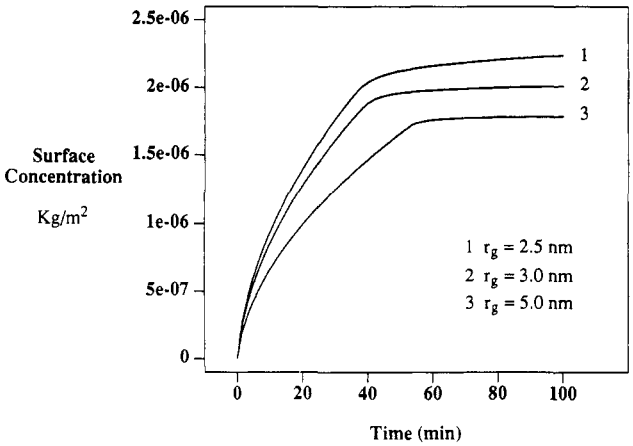

Figure 9. Effect of size of a spherical protein molecule on the evolution of surface concentration with time; h_s = 6 × 10^{-21} J/residue, n_s = 1.55 × 10^{18} J/residue, and c_b = 2 × 10^{-4} wt %.

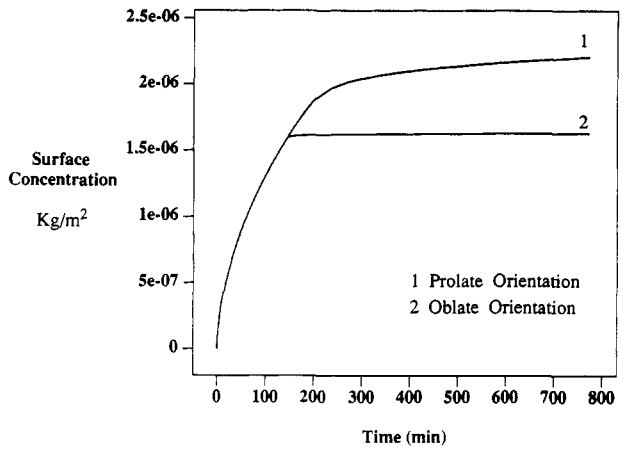

Figure 10. Effect of orientation of an ellipsoidal protein molecule on the variation of its surface concentration with time; protein size 8.5 × 1.65 × 1.65 nm, h_s = 1 × 10^{-21} J/residue, n_s = 1.55 × 10^{18} residues/m^2, and c_b = 2 × 10^{-4} wt %. Curves 1 and 2 refer to prolate and oblate orientations of the protein molecule, respectively.

adsorption. This is due to the fact that an increase in the minor axis leads to an increase in the energy barrier for adsorption, leading to lower surface concentration. In addition, as the minor axis increases, the size of the protein molecule increases and hence the diffusion coefficient decreases. As a result, the larger protein molecules diffuse more slowly than the smaller ones and therefore take longer

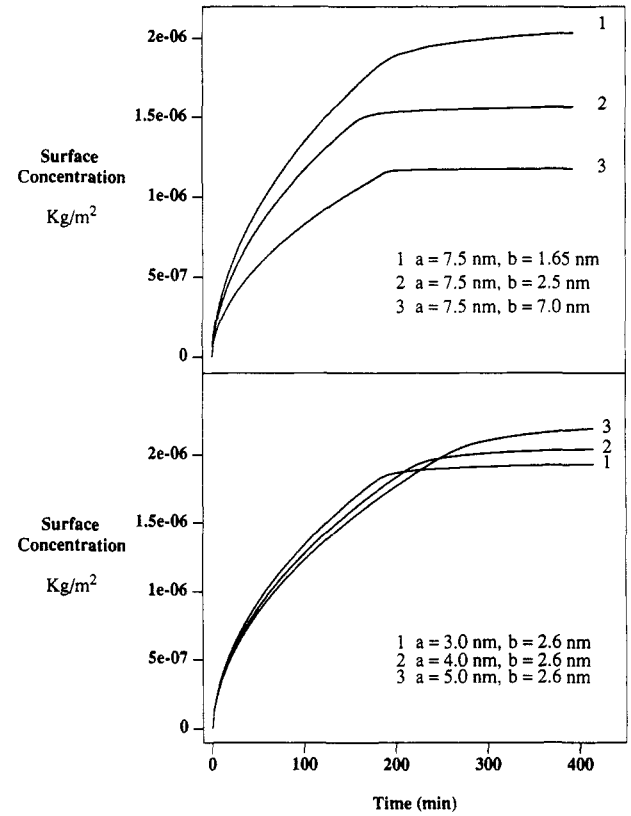

Figure 11. Effect of change in the minor (top panel) and major (bottom panel) axes of a prolate protein molecule on kinetics of adsorption; c_b = 2 × 10^{-4} wt %, n_s = 1.55 × 10^{18} residues/m^2, and h_s = 10 × 10^{-21} J/residue (top panel) or h_s = 4.5 × 10^{-21} J/residue (bottom panel).

to adsorb. In contrast, even though an increase in the major axis of the protein molecule leads to lower diffusion coefficients, the contribution of hydrophobicity to the interaction energy of the protein molecule increases. This leads to a lower energy barrier and hence a higher driving force for adsorption. These two effects oppose each other and hence we see a crossover of the surface concentration–time curves as the size of the major axis increases.

The concentration profile of globular protein in region II was evaluated from eqs 26 and 27. A typical evolution of the profile in region II is shown in Figure 12 as a plot of dimensionless concentration versus distance at various times. Since region I is very much smaller than region II, the variation of concentration within region I cannot be shown in the figure. Initially, the protein concentration is uniform. At short times, the dimensionless protein concentration η^* at the interface of regions I and II is zero because adsorption is diffusion controlled. At larger times, however, η^* is nonzero because of the resistance to adsorption in region I due to the energy barrier. Of course, η^* increases with time due to increased resistance to adsorption in region I (larger energy barrier) as the surface concentration builds up. At sufficiently large times (large surface concentrations), the energy barrier to adsorption is so high that all the resistance to adsorption lies in region I so that the concentration profile in region II is more or less uniform.

The predicted evolution of surface concentration with time for adsorption of lysozyme at an air–water interface at a pH of 7 and an ionic strength of 0.1 for two extreme orientations of the ellipsoidal molecule (prolate and oblate) are compared with the experimental data (Graham and Phillips, 1979a) in Figure 13. Lysozyme is an ellipsoidal molecule of dimensions 4.5 × 3 × 3 nm. Since lysozyme

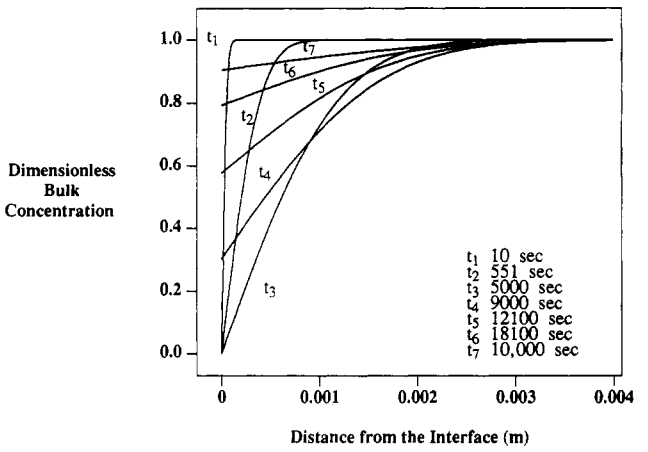

Figure 12. Typical dimensionless concentration profile in region II at different times; protein size 7.5 × 3.0 × 3.0 nm, $h_s = 1 \times 10^{-21}$ J/residue, $n_s = 1.55 \times 10^{18}$ residues/m^2, and $c_b = 2 \times 10^{-4}$ wt %.

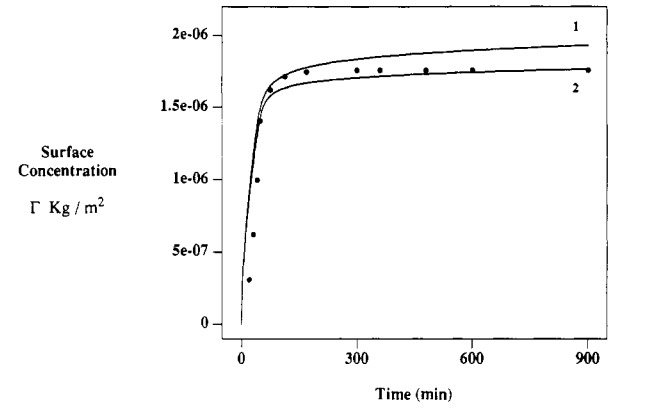

Figure 13. Comparison of model predictions with experimental data for lysozyme (●) at pH 7 [charge on lysozyme 8.8 (Devereux, 1984)], protein size 4.5 × 3.0 × 3.0 nm (Blake et al., 1965), and diffusivity 8×10^{-10} m^2/s (Graham et al., 1979a). Curves 1 and 2 refer to prolate and oblate orientations of the protein molecules, respectively.

is charged at pH 7 (Devereux, 1984), the electrical energy barrier for adsorption was evaluated from eqs 3–6. The diffusion coefficient of lysozyme is taken to be 8×10^{-10} m^2/s as determined by Graham and Phillips (1979a) from their experimental data of kinetics of adsorption at short times. In order to evaluate the surface hydrophobicity, the fraction of hydrophobic residues that lie on the surface should be known. If V_i is the volume of the residues that lie buried inside the protein molecule and V_e is the volume of the residues that lie on the surface exposed to the aqueous phase, then the fraction ϕ_{aq} of the residues that lie on the surface is given by

$$\phi_{aq} = \frac{V_e}{V_i + V_e} = \frac{p}{1+p} \tag{31}$$

where $p = V_e/V_i$. For lysozyme, the value of p is 1.18 (Bigelow, 1967). Since the total number of amino acid residues is 147, the number of residues that lie on the surface turns out to be 80. At pH 7 there are 40 charged amino acid residues in lysozyme (Devereux, 1984). Assuming that all the charged residues lie on the surface, the number of hydrophobic residues that lie on the surface is 40. Therefore, the surface hydrophobicity h_s is given by

$$h_s = \frac{40}{147-40}h \tag{32}$$

where h is the hydrophobicity arising from all the hydrophobic residues and is 970 cal/mol of residues (Graham and Phillips, 1979a) for lysozyme. Therefore, h_s for lysozyme is 362 cal/mol of residues. As can be seen from Figure 13, the experimental data lie between the predicted kinetics of adsorption for two extreme orientations. It is to be noted that the proposed model is valid only for monolayer adsorption of globular proteins at an air–water interface. In case of multilayer adsorption, the interaction potential has to be modified since the protein molecule does not penetrate the interface and therefore does not have to do work against the surface pressure.

4. Conclusions

A model for the kinetics of adsorption of globular proteins at an air–water interface is proposed. Adsorption of globular protein at an air–water interface from an infinite stagnant medium is considered as diffusion in a potential field. The interaction potential experienced by an adsorbing protein molecule consisted of contributions from hydrophobic and electrostatic interactions as well as the work done against the surface pressure to clear a certain area to anchor the adsorbed segments at the interface. Comparison of the energy barriers to adsorption and desorption indicated that the rates of desorption are likely to be very small, thus justifying the assumption of irreversible adsorption. In addition to the size and shape of the molecule, the interaction potential depends on surface hydrophobicity, surface pressure, pH, ionic strength, and orientation of a nonspherical protein molecule. At sufficiently large surface pressures, an adsorbing globular protein molecule has been shown to experience an energy barrier. The energy barrier is found to be higher for smaller surface hydrophobicities, larger surface pressures, and larger size molecules. In the case of ellipsoidal protein molecules, the energy barrier is found to be higher for an oblate orientation than prolate, thus making the latter orientation more favorable. Calculation of the evolution of surface concentration of globular protein at an air–water interface with time for different parameters indicated that more adsorption occurred at higher bulk concentrations, larger surface hydrophobicities, and smaller size. At small times, the kinetics of adsorption of ellipsoidal protein molecules was found to be independent of orientation since adsorption was diffusion controlled. At larger times, however, more adsorption occurred for prolate than oblate orientation since the energy barrier for adsorption was smaller for the former than the latter. The subphase concentration (concentration at the interface of regions I and II) has been shown to be zero at short times, to increase with time at larger times, and eventually to become close to the bulk concentration. The predicted evolution of surface concentration with time for adsorption of lysozyme at an air–water interface at a pH of 7 and an ionic strength of 0.1 agreed well with the experimental data of Graham and Phillips (1979a).

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Appendix

For a prolate or oblate protein molecule, shown in the middle and bottom panels of Figure 1, respectively, when less than half the protein molecule has penetrated through the interface, the area A_r required to anchor at the gas–

liquid interface is given by the expression

$$A_r = \int_{x_1}^{x_2}\left[1 - \frac{x^2}{b^2\left(1-\left(\frac{a-h_t}{a}\right)^2\right)}\right]^{1/2} \times a\left[1-\left(\frac{a-h_t}{a}\right)^2\right]^{1/2} dx \quad \text{(A1)}$$

where

$$x_{1,2} = \pm b\left[1-\left(\frac{a-h_t}{a}\right)^2\right]^{1/2}$$

When more than half the protein molecule has penetrated the interface, the area A_r is given by

$$A_r = \int_{x_1}^{x_2} a\left(1-\frac{x^2}{b^2}\right) dx \quad \text{(A2)}$$

where

$$x_{1,2} = \pm b$$

For the spherical protein molecule shown in the top panel of Figure 1, $a = b = r_g$, and when less than half of the protein molecule has penetrated in the interface

$$A_r = \pi r_g^2\left[1-\left(\frac{r_g-h_t}{r_g}\right)^2\right] \quad \text{(A3)}$$

When more than half of the spherical protein molecule has penetrated in the interface

$$A_r = \pi r_g^2 \quad \text{(A4)}$$

For an ellipsoidal protein molecule (Figure 1, middle and bottom panels), the surface area that is exposed for less than half the penetration of the protein molecule above the interface A_s is given by the expression

$$A_s = 2\pi\int_{x_1}^{x_2} f(x)(1 + (f'(x))^2)^{1/2}\, dx \quad \text{(A5)}$$

where $f(x) = a[1-(x^2/b^2)]^{1/2}$ is the equation of the ellipse in the x–y plane, $x_1 = a - h_t$, and $x_2 = a$. When the penetration of the protein molecule is more than half, then the surface area is given by

$$A_s = 2\pi\left[\int_{x_1}^{x_2} f(x)(1 + (f'(x))^2)^{1/2}\, dx + \int_{x_3}^{x_4} f(x)(1 + (f'(x))^2)^{1/2}\, dx\right] \quad \text{(A6)}$$

where $x_1 = x_3 = 0$, $x_2 = a$, and $x_4 = h_t$.

For a spherical protein molecule, $a = b = r_g$ and $f(x) = r_g[1 - (x^2/r_g^2)]^{1/2}$ is the equation of a circle in the x–y plane.

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