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Research Article

A KINETIC MODEL FOR MONO-LAYER GLOBULAR PROTEIN ADSORPTION ON SOLID/LIQUID INTERFACES

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Abstract: A kinetic model was derived for globular protein adsorption. The model takes into account the three possible scenarios of a protein molecule in solution, being exposed to an interface: adsorption step from the solution to the interface; the possible desorption back into the solution; and the surface-induced unfolding or spreading of the protein unto the substrate surface. A globular protein molecule is visualized as a sphere with radius D. In addition to the general case of protein adsorption, which portrays either the surface coverage (θ) or surface concentration (Γ) as a function of the adsorption time, special cases, like equilibrium condition, low-surface coverage, irreversible, and Langmuirian were also presented and treated in light of the derived model. The general model was simplified for each of the subset cases. The irreversibility versus reversibility of protein adsorption was discussed. The substrate surface energetics or effects are accounted for via the proposition of the percent relative change in D/V ratio for the adsorbing protein, called $(D/V)_{PRC}$ parameter. $(D/V)_{PRC}$ is calculated with respect to the monolayer surface concentration of protein, where the latter is given by β/α ratio. This can be used as a landmark to protein adsorption isotherms or even kinetics. This is visualized as an indicator for solid substrate effects on the adsorbing proteins. $(D/V)_{PRC}$ can be zero (fresh monolayer), negative (aged monolayer), or positive (multi-layer). The reference surface concentration is reported for some selected proteins.

Keywords: Protein adsorption; kinetic model; unfolding; surface coverage; globular protein; protein desorption; denaturation.

Introduction

Protein adsorption is involved in a number of areas in biology, medicine, food and pharmaceutical processing, and biotechnology. In the food and pharmaceutical industries, proteins can play a major role in the fouling of membrane surfaces used in bio-molecular fractionation, and fouling of heat exchange surfaces due to their heat sensitivity and high content in some fluid foods. Additionally, protein behavior at both air/water and oil/water interfaces can play a major role in stabilizing colloidal food systems, foams, and emulsions. The arrival of protein at the interface during the early stages of the process is mainly transport limited (MacRitchie and Alexander, 1963). In the later stages, rate of adsorption is less than that predicted by the diffusion-controlled rate merely due to the existence of kinetic barriers at the interface; i.e., occupancy or steric effects (Graham and Philips, 1979; Damodaran and Song, 1988).

In general, adsorption involves migration of a substance from one phase to the surface of an adjacent phase, accompanied by its accumulation at the interface (Slejko, 1985). Adsorption is a result of the binding forces between individual atoms, ions, or molecular regions of an adsorbate and the adsorbent surface. These binding forces or interactions vary in magnitude down from the weak van der Waals-type of attractions contributing to physical adsorption, up to the

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strong covalent bonds in chemisorption. Polymer adsorption and biopolymer adsorption both show a range of binding energies depending on the type of forces present in the interface.

Once adsorbed, proteins can undergo varying levels of orientational and conformational change, resulting in a change both in the monolayer coverage and in the binding strength (interaction energy) between the substrate interface and adsorbing protein. This simply means that adsorbing molecules may exist in more than one adsorbed state (Lundström, *et al.*, 1987; Horbett and Brash, 1987) each of which is characterized by its binding strength, orientation with respect to the interface, and geometrical shape and molecular size. Of course, one should recall the fact that the existence of multiple adsorbed states is not merely due to adsorbate-adsorbent interactions but also extends to lateral, adsorbate-adsorbate interactions, where both effects give rise to a time-, protein-, and surface-dependent protein unfolding.

Van der Veen *et al.* (2007) examined the kinetics of adsorption of lysozyme and a-lactalbumin from aqueous solution on silica and hydrophobized silica. The initial rate of adsorption of lysozyme at the hydrophilic surface was found to be comparable with the limiting flux (function of hydrodynamic condition, the concentration, and the diffusion coefficient of the protein). On the other hand, they found that for lysozyme at the hydrophobic surface and a-lactalbumin on both surfaces, the rate of adsorption was lower than the limiting flux, but the adsorption proceeded cooperatively. They found that at the hydrophilic surface, adsorption saturation of both proteins strongly depended on the rate of adsorption, but for the hydrophobic surface no such dependency was observed. They attributed that to the structural relaxation ("spreading") of the adsorbed protein molecules, which occurs at the hydrophobic surface faster than at the hydrophilic one. For lysozyme, desorption has been studied as well. It was also found that the desorbable fraction decreased after longer residence time of the protein at the interface.

Fainerman *et al.* (2006) examined the adsorption kinetics of proteins at the solution/air interfaces with controlled bulk convection. The adsorption kinetics of beta-casein, beta-lactoglobulin, and HSA were studied at solution/gas interface with and without forced convection. It was shown that, in spite of a significant difference in the adsorption rates for these two experiments by one order of magnitude, the equilibrium surface tension values were virtually the same. That led them to the conclusion about the thermodynamic reversibility of protein adsorption at the solution/gas interface, in contrast to the kinetic irreversibility of the process, which was highly pronounced at solid/liquid interfaces. They attributed such a behavior possibly to the fact that the re-conformation of a protein molecule at air surface was comparatively rapid. The time characteristic for the re-conformation process was essentially lower than the time necessary to attain the equilibrium state in experiments with forced convection.

Imamur *et al.* (2010) examined the influences of properties of protein and adsorption surface on removal kinetics of protein adsorbed on metal surface by H_2O_2-electrolysis treatment. They found that the rate of removal of an adsorbed protein appeared to be strongly dependent on the adsorptive force, which would correspond to the sum of the pairwise adsorptive interactions formed by a single adsorbed protein molecule. As the pH during the adsorption was lower, and/or the isoelectric point (pI) of a metal surface was higher, a larger number of the electrostatic interactions between protein $-COO^-$ groups and $-OH_2^+$ groups on a metal surface were formed. This resulted in a strong adsorption and thus lower removal rates in cases of acidic and weakly basic proteins. On the other hand, for basic proteins, there were a significant repulsion between the surface of the protein and that of the metal at extreme pH values (i.e., highly acidic or highly basic). Hence, the removal rates were high at such extremities and were low at pH's equal to or slightly lower than pI of the metal surface.

Wierenga *et al.* (2006) examined whether the folding state of the adsorbed protein depended on the rate of adsorption to the interface, which could be controlled by bulk concentration. Subsequently, the adsorption of proteins with varying structural stabilities at several protein

concentrations was studied using ellipsometry and surface tensiometry. For β-lactoglobulin, the adsorbed amount (γ) needed to reach a certain surface pressure (π) decreased with decreasing bulk concentration. Ovalbumin, on the other hand, showed no such dependence. To verify whether this difference in behavior was caused by the difference in structural stability, similar experiments were performed with cytochrome **c** and a destabilized variant of it. Both proteins showed identical π–γ, and no dependence on bulk concentration. They concluded that unfolding would only take place if the kinetics of adsorption was similar or slower than the kinetics of unfolding. The latter was found to depend on the activation energy of unfolding (which is in the order of 100–300 kJ/mol), rather than on the free energy of unfolding (typically 10–50 kJ/mol).

In this work, a theoretical kinetic model is developed while taking into account that adsorbing protein molecule is subject to surface-induced unfolding, in addition to the typical adsorption and desorption events.

Model Derivation

Writing the component material balance (i.e., mass action law) for protein, one gets:

Rate of adsorption – Rate of desorption = Rate of accumulation (1)

Rate of adsorption: $k_a C_o (1 - \theta) A$

where k_a is adsorption rate constant [m/s]; C_o the protein bulk concentration [mole/m^3]; θ the fractional surface coverage; and A the total surface area [m^2] available for adsorption.

Rate of desorption: $k_d \theta A \Gamma$

where k_d is desorption rate constant [s^{-1}]; and Γ the protein surface concentration [mole/m^2].

Rate of accumulation: $\frac{d(\theta \Gamma A)}{dt}$

Thus Eq. (1) becomes:

$$k_a C_o (1-\theta) A - k_d \theta A \Gamma = \frac{d(\theta \Gamma A)}{dt} \qquad (2)$$

The total surface area, A, can be factored out, for A of a typical solid/liquid interface has fixed geometries. Consequently, Eq. (2) reduces to:

$$k_a C_o (1-\theta) - k_d \theta \Gamma = \frac{d(\theta \Gamma)}{dt} \qquad (3)$$

The accumulation term, which appears in Eq. (3), can be further simplified into:

$$\frac{d(\theta \Gamma)}{dt} = \theta \frac{d(\Gamma)}{dt} + \Gamma \frac{d(\theta)}{dt}$$

Thus, Eq. (3) becomes:

$$k_a C_o (1-\theta) - k_d \theta \Gamma = \theta \frac{d(\Gamma)}{dt} + \Gamma \frac{d(\theta)}{dt} \qquad (4)$$

From cited literature (Damodaran and Song, 1988; Andrade, 1985; Macritchie, 1978; Ter-Minassian-Saraga, 1981; Al-Malah, 1993) it is known that the protein molecule approaching the surface needs only a minimal surface area requirement (i.e., a foothold) so that it anchors itself to the adsorption site. As time passes and based on conformational stability of adsorbing protein and the extent of fractional surface coverage, θ, surface-induced unfolding takes place, which in turn alters the surface concentration of protein molecule, Γ. On the other hand, neighboring molecules that are less tenaciously bound to the interface, are adversely affected and eventually desorb (go back) to the solution.

The first term that appears on the right hand side of Eq. (4) mainly accounts for the slow rate of surface-induced protein unfolding (or denaturation), which is manifested via the change in surface concentration, Γ, with time; whereas, the second term mainly accounts for the net rate of filling (occupying) vacant adsorption sites. Proteins are, in general, rod-type or globular in shape. In a previous work (Al-Malah and Mousa, 2002), visualization of protein molecule as being cylindrical (or ellipsoidal) in shape (with the height of the cylinder being the major axis of protein molecule and the diameter as the minor axis) has been proposed and was presented in details. Here, visualization of protein molecule as being spherical (globular) is assumed. The fractional surface coverage, θ, can be defined as:

$$\theta = \frac{N_S \pi D^2}{A} \qquad (5)$$

where N_s is the total number of adsorbing molecules and D is the diameter [m] of the sphere. The total number of adsorbing molecules, N_s, can be further expressed as:

$$N_S = \frac{n_P V_P}{\frac{\pi D^3}{6}} \tag{6}$$

where n_P [mole] is the number of moles of adsorbing protein, V_P [m^3/mole] is the molar volume of adsorbing protein, and $\frac{\pi D^3}{6}$ is the molecular volume [m^3/molecule] of adsorbing protein. Substituting the value of N_s (Eq. 6) in Eq. (5), θ becomes:

$$\theta = \frac{n_P V_P}{\frac{\pi D^3}{6}} \times \frac{\pi D^2}{A} = 6 \times \left(\frac{n_P}{A}\right) \times \left(\frac{V_P}{D}\right) = 6\Gamma \times \left(\frac{V_P}{D}\right)_{interface} \tag{7}$$

Strictly speaking, the molecular properties appearing in $\left(\frac{V_P}{D}\right)_{interface}$ ratio should be evaluated at the inter-phase not at the bulk-phase conditions and should be adsorption-time-dependent. However, the $\left(\frac{V_P}{D}\right)_{interface}$ ratio will be assumed constant over the time course of adsorption. As will be further explained, both values of $\left(\frac{V_P}{D}\right)$ ratio (i.e., in solution and at the interface) will be contrasted; $\left(\frac{V_P}{D}\right)_{solution}$ will be determined based on the molecular dimensions of a given protein in solution; on the other hand, $\left(\frac{V_P}{D}\right)_{interface}$ will be calculated based on the computer-generated, regressed parameters belonging to the existing kinetic model, namely α and β. Difference in values of $\left(\frac{V_P}{D}\right)$ ratio will serve as an indication of the extent of surface-induced unfolding of adsorbing protein or the presence of a multilayer film. For cosmetic reasons and to make equations a more plausible, from now on, the subscription will be dropped out, with the understanding that $\left(\frac{V_P}{D}\right)$ really means $\left(\frac{V_P}{D}\right)_{interface}$.

Eliminating the value of θ in favor of Γ using Eq. (7), Eq. (4) becomes:

$$k_a C_o (1-\theta) - k_d \theta \Gamma = \theta \frac{d(\Gamma)}{dt} + \Gamma \frac{d\left(6\Gamma \frac{V_P}{D}\right)}{dt} = \theta \frac{d(\Gamma)}{dt} + 6\Gamma \frac{V_P}{D} \frac{d(\Gamma)}{dt} \tag{8}$$

It is not difficult to realize that the second term on the right-hand side of Eq. (8) is equal to $\theta \frac{d\Gamma}{dt}$, therefore, Eq. (8) becomes:

$$k_a C_o (1-\theta) - k_d \theta \Gamma = \theta \frac{d(\Gamma)}{dt} + \theta \frac{d(\Gamma)}{dt} = 2\theta \frac{d(\Gamma)}{dt} \tag{9}$$

Dividing through by θ, and rearranging, Eq. (9) becomes:

$$k_a C_o \left(\frac{1-\theta}{\theta}\right) - k_d \Gamma = 2\frac{d(\Gamma)}{dt} \tag{10}$$

Eliminating θ in favor of Γ using Eq. (7), Eq. (10) becomes:

$$k_a C_o \left(\frac{D}{6V_P \Gamma} - 1\right) - k_d \Gamma = 2\frac{d(\Gamma)}{dt} \tag{11}$$

Multiplying through by Γ yields:

$$k_a C_o \frac{D}{6V_P} - k_a C_o \Gamma - k_d \Gamma^2 = 2\Gamma \frac{d(\Gamma)}{dt} \tag{12}$$

Eq. (12) obviously is a non-linear ordinary differential equation. To facilitate the solution of Eq. (12), the following notations will be adopted:

$$\beta = k_a C_o \frac{D}{6V_P} \tag{13}$$

$$\alpha = k_a C_o \tag{14}$$

$$y = \Gamma;\ x = t \tag{15}$$

$$y' = \frac{dy}{dx} = \frac{d\Gamma}{dt} \tag{16}$$

Consequently, Eq. (12) becomes:

$$\beta - \alpha y - k_d y^2 = 2yy' = 2y\frac{dy}{dx} \tag{17}$$

Upon re-arrangement of Eq. (17), it reduces to:

$$dx = \frac{2ydy}{\beta - \alpha y - k_d y^2} \tag{18}$$

Alternatively, in an integral notation, it becomes:

$$\int dx = \int \frac{2ydy}{\beta - \alpha y - k_d y^2} \tag{19}$$

From calculus (Spiegel, 1968), it is given that:

$$\int \frac{ydy}{(ay+b)(py+q)} = \frac{1}{bp-aq}\left[\frac{b}{a}ln(|ay+b|) - \frac{q}{p}ln(|py+q|)\right] \tag{20}$$

If the denominator, $\beta - \alpha y - k_d y^2$, is matched with the standard form, $(ay + b)(py + q)$ of Eq. (20), then the following equalities are obtained:

$$a = 1 \tag{21a}$$

$$p = 1 \tag{21b}$$

$$b = \frac{\alpha + \sqrt{\alpha^2 + 4k_d\beta}}{2k_d} \tag{21c}$$

$$q = \frac{\alpha - \sqrt{\alpha^2 + 4k_d\beta}}{2k_d} \tag{21d}$$

If dx, which is dt, is integrated from *zero*, as the lower limit, to any value x, as the upper limit, then dy, which is $d\Gamma$, will be integrated from *zero*, as the lower limit, to any value y, as the upper limit. Therefore, using Eq. (20) while making use of equalities found in equations (21a) through (21d), the final solution of Eq. (17) will be:

$$x = \left[\frac{\alpha + \sqrt{\alpha^2 + 4k_d\beta}}{\sqrt{\alpha^2 + 4k_d\beta}} ln\left(\left|y + \frac{\alpha + \sqrt{\alpha^2 + 4k_d\beta}}{2k_d}\right|\right)\right] - \left[\frac{\alpha - \sqrt{\alpha^2 + 4k_d\beta}}{\sqrt{\alpha^2 + 4k_d\beta}} ln\left(\left|y + \frac{\alpha - \sqrt{\alpha^2 + 4k_d\beta}}{2k_d}\right|\right)\right] \tag{22}$$

If x is replaced by t; and y by Γ, then Eq. (22) becomes:

$$t = \left[\frac{\alpha + \sqrt{\alpha^2 + 4k_d\beta}}{\sqrt{\alpha^2 + 4k_d\beta}} ln\left(\left|\Gamma + \frac{\alpha + \sqrt{\alpha^2 + 4k_d\beta}}{2k_d}\right|\right)\right] - \left[\frac{\alpha - \sqrt{\alpha^2 + 4k_d\beta}}{\sqrt{\alpha^2 + 4k_d\beta}} ln\left(\left|\Gamma + \frac{\alpha - \sqrt{\alpha^2 + 4k_d\beta}}{2k_d}\right|\right)\right] \tag{23}$$

Eq. (23) is the solution for the general case (Eq. 10 or 12). It implicitly gives the surface concentration, γ, as a function of adsorption time. There are, however special cases of Eq. (10) which will be presented shortly.

CASE A: A Steady-State (i.e. Equilibrium) Surface Concentration

Notice that for the steady-state (i.e., equilibrium) value of Γ, while $k_d \neq 0$, the right-hand side of Eq. (17) is set to zero. This yields:

$$\beta - \alpha y - k_d y^2 = 0 = \beta - \alpha\Gamma_{eq} - k_d\Gamma_{eq}^2 = 0 \tag{24}$$

This is a quadrature and the solution of which is given by:

$$\Gamma_{eq} = \frac{\alpha \mp \sqrt{\alpha^2 + 4k_d\beta}}{-2k_d} \qquad k_d \neq 0 \tag{25}$$

Since Γ is always a positive and real quantity, we retain the positive real root of Eq. (25). Hence, we have:

$$\Gamma_{eq} = \frac{\alpha - \sqrt{\alpha^2 + 4k_d\beta}}{-2k_d} = \frac{\sqrt{\alpha^2 + 4k_d\beta} - \alpha}{2k_d} \quad k_d \neq 0 \tag{26}$$

On the other hand, if k_d is set to zero (i.e., irreversible protein adsorption), then we have from Eq. (17):

$$\beta - \alpha y = 0 = \beta - \alpha\Gamma_{eq} = 0 \rightarrow \Gamma_{eq} = \frac{\beta}{\alpha} = \frac{1}{6} \times \left(\frac{D}{V_P}\right)_{int\,erface} \quad k_d = 0 \tag{27}$$

Eq. (26) allows the calculation of the equilibrium surface concentration, Γ_{eq}, in terms of the adsorption rate constant, the bulk concentration, desorption rate constant, and molecular properties of adsorbing protein. On the other hand, Eq. (27) allows the calculation of the equilibrium surface concentration, Γ_{eq}, in terms of the molecular properties of adsorbing protein.

CASE B: Low Surface Coverage

If the surface coverage, θ, is relatively small (i.e., θ<<1.0), then Eq. (10) can be simplified as follows:

$$k_\alpha C_o\left(\frac{1}{\theta}\right) - k_d\Gamma = 2\frac{d(\Gamma)}{dt} \tag{28}$$

Rearranging Eq. (28) while recalling that $\left(\theta = 6\Gamma \times \left(\frac{V_P}{D}\right)\right)$, one obtains the following equation:

$$\frac{d(\Gamma)}{dt} + \frac{k_d}{2}\Gamma = \frac{k_a C_o D}{12V_P}(\Gamma^{-1}) \tag{29}$$

Eq. (29) is Bernoulli's equation that can be transformed into a first-order linear ordinary differential equation via the following transformations:

$$\Gamma^* = \Gamma^2 \tag{30a}$$

$$\frac{d\Gamma^*}{dt} = 2\Gamma\frac{d\Gamma}{dt} \tag{30b}$$

Utilizing Eq. (30b), Eq. (29) becomes:

$$\frac{d(\Gamma^*)}{dt}\frac{1}{2}\Gamma^{-1} + \frac{k_d}{2}\Gamma = \frac{k_a C_o D}{12V_P}(\Gamma^{-1}) \tag{31}$$

Multiplying Eq. (31) by 2Γ, it becomes:

$$\frac{d(\Gamma^*)}{dt} + k_d\Gamma^2 = \frac{k_a C_o D}{6V_P} \tag{32}$$

Upon utilizing Eq. (30a), Eq. (32) becomes:

$$\frac{d(\Gamma^*)}{dt} + k_d\Gamma^* = \frac{k_a C_o D}{6V_P} \tag{33}$$

Eq. (33) is now a first-order linear ordinary differential equation, which has the following solution:

$$\Gamma^* e^{k_d t} = \int \frac{k_a C_o D}{6V_P} e^{k_d t} dt + C \tag{34}$$

where *C* is the integration constant.

Upon integration of the first term on the right hand side of Eq. (34) and dividing through by the exponential term, Eq. (34) becomes:

$$\Gamma^* = \frac{k_a C_o D}{6V_P k_d} + Ce^{-k_d t} \tag{35}$$

Given that at $t = 0$, $\Gamma^* = 0$, allows us to evaluate the constant of integration, *C*. Therefore, Eq. (35) becomes:

$$\Gamma^* = \frac{k_a C_o D}{6V_P k_d} \times (1 - e^{-k_d t}) \tag{36}$$

Upon back substitution of Γ^* *by* Γ^2, Eq. (36) becomes:

$$\Gamma = \sqrt{\frac{k_a C_o D}{6V_P k_d} \times (1 - e^{-k_d t})} = \sqrt{\frac{\beta}{k_d} \times (1 - e^{-k_d t})} \tag{37}$$

CASE C: Irreversible Adsorption (k_d = 0)

In this case, with k_d=*0*, Eq. (10) becomes:

$$k_a C_o\left(\frac{1-\theta}{\theta}\right) = k_a C_o\left(\frac{1}{\theta}\right) - k_a C_o = 2\frac{d(\Gamma)}{dt} \tag{38a}$$

Substituting the value of $\left(\theta = 6\Gamma \times \left(\frac{V_P}{D}\right)\right)$ in Eq. (38a) yields:

$$k_\alpha C_o\left(\frac{D}{6V_P\Gamma}\right) - k_a C_o = 2\frac{d(\Gamma)}{dt} \tag{38b}$$

Noting that $\beta = k_a C_o \frac{D}{6V_P}$ and $\alpha = k_a C_o$ and multiplying by Γ throughout, Eq. (38b) becomes:

$$\beta - \alpha\Gamma = 2\Gamma\frac{d\Gamma}{dt} \tag{39}$$

The solution of Eq. (39), given that at $t = 0$, $\Gamma = 0$, is:

$$t = \frac{2}{\alpha^2}\left[\beta ln\left(\frac{\beta}{\beta - \alpha\Gamma}\right) - \alpha\Gamma\right] \tag{40a}$$

If Taylor series expansion is made to linearize the natural logarithm term (see Appendix), the following approximation is obtained:

$$t = \frac{2}{\alpha^2}\left[\beta\frac{\alpha\Gamma}{\beta - \alpha\Gamma} - \alpha\Gamma\right] = \frac{2}{\alpha^2}\left[\frac{\alpha\beta\Gamma}{\beta - \alpha\Gamma} - \alpha\Gamma\right] = \frac{2}{\alpha}\left[\frac{\beta\Gamma}{\beta - \alpha\Gamma} - \Gamma\right] \tag{40b}$$

CASE D: Langmuir Model $\left(\frac{d\Gamma}{dt} = 0\right)$

The right hand side of Eq. (10) reduces to zero, whereas all variables assume their equilibrium values; hence, Eq. (10) becomes:

$$k_\alpha C_{eq}\left(\frac{1 - \theta_{eq}}{\theta_{eq}}\right) - k_d\Gamma_{eq} = 0 \tag{41}$$

Denoting (k_a/k_d) by K_{eq} and solving for θ_{eq} one gets:

$$\theta_{eq} = \frac{K_{eq}C_{eq}}{\Gamma_{eq} + K_{eq}C_{eq}} \tag{42}$$

Alternatively, it can be expressed as:

$$\theta_{eq} = \frac{C_{eq}}{(\Gamma_{eq}/K_{eq}) + C_{eq}} \tag{43}$$

For a sufficiently large value of $C_{eq}\left(i.e., C_{eq} >> \left(\Gamma_{eq}/K_{eq}\right)\right)$, θ_{eq} approaches one, which implies a complete monolayer surface coverage.

On the other hand, for a sufficiently small value of $C_{eq}\left(i.e., C_{eq} << \left(\Gamma_{eq}/K_{eq}\right)\right)$, θ_{eq} is given by:

$$\theta_{eq} = \frac{K_{eq}}{\Gamma_{eq}}C_{eq} \tag{44}$$

Eq. (44) gives a straight line relationship which represents the initial slope of a typical adsorption isotherm.

Discussion

Having a look at Eq. (23), which represents the general case for protein adsorption kinetics (Γ vs. t), will reveal that there are three parameters which will portray the picture of protein adsorption kinetics, namely, α, β, and k_d. It is hypothesized here that protein adsorption will be more likely to be reversible during the initial events (i.e., within a scale of a second or even less). After that, the protein adsorption will become irreversible, or at least to say that the adsorption rate constant is way larger than the desorption rate constant. Strictly speaking, all reactions are reversible; however, when the forward rate constant is much larger than the backward rate constant, thence we spell out the reaction to be irreversible in chemical reaction engineering or in reaction chemistry (kinetics) terminology.

Unless an experiment is precisely devised to monitor the protein adsorption on the molecular (nano-scale) level during the initial events, it is really hard to pick up the effect of desorption on the course of protein adsorption. Nevertheless, the door will be left open for future experiments, techniques, and setups that will be capable to analyze and diagnose protein adsorption on the molecular level while keeping both rate constants an order of magnitude the same. It is worth mentioning here what Aptel *et al.* (1988) had already reported that the change in the affinity constant k_a/k_d was mainly due to the change in the adsorption constant, k_a.

Hence, we are left with two parameters that will govern the extent of protein adsorption; they are α and β. For a typical protein adsorption experiment, whether it is carried out as a function of bulk concentration (isotherm) or as a function of adsorption time (kinetics), the generic plot (prototype) is such that the curve is characterized by an initial slope followed by either a plateau (monolayer) or multi-step plateau (multilayer). However, for some experiments it may not look as a multi-step plateau but continuously increases over either time or equilibrium bulk concentration (Freundlich as opposed to Langmuir behavior). At a given bulk concentration, the value of the initial slope will depict the term α and the plateau or the plateau-like will depict the parameter β.

As pointed out earlier in Model Derivation section, difference in values of $\frac{D}{V_P}$ ratio will serve as an indicator of either the extent of surface-induced unfolding of adsorbing protein

$$\left(i.e., \frac{\beta}{\alpha} = \frac{1}{6} \times \left(\frac{D}{V_P} \right)_{interface} < \frac{1}{6} \times \left(\frac{D}{V_P} \right)_{solution} \right) \quad (45)$$

Or, the presence of a multilayer film:

$$\left(i.e., \frac{\beta}{\alpha} = \frac{1}{6} \times \left(\frac{D}{V_P} \right)_{interface} > \frac{1}{6} \times \left(\frac{D}{V_P} \right)_{solution} \right) \quad (46)$$

Recalling Eq. (27) one may recognize that the equilibrium surface concentration, Γ_{eq}, under irreversible condition (k_d = 0), reduces to:

$$\Gamma_{eq} = \frac{\beta}{\alpha} = \frac{1}{6} \times \left(\frac{D}{V_P} \right)_{interface} \quad [mole/m^2] \quad (27)$$

Eq. (27), to a large extent, corresponds to the monolayer surface concentration of protein. This can be used as a reference or landmark to protein adsorption isotherms or even kinetics. Values higher than or less then this value will indicate the effect of substrate surface effects or energetics. In other words, surface-induced unfolding (conformational change) or multilayer film formation will respectively dictate a negative or positive deviation from such a nominal value. One more thing to notice is that the reciprocal value of $\left(\frac{D}{V_P} \right)_{interface}$ is essentially the molar surface area occupied by one mole of adsorbing protein molecules.

Alternatively, the following parameter can also be used to indicate such interfacial effects:

$$(D/V)_{Percent\ Relative\ Change} = (D/V)_{PRC} = \frac{\left(\frac{D}{V_P} \right)_{interface} - \left(\frac{D}{V_P} \right)_{solution}}{\left(\frac{D}{V_P} \right)_{solution}} \times 100\% \quad (47)$$

Or,

$$(D/V)_{PRC} = \frac{(\Gamma)_{interface} - (\Gamma)_{solution\,(i.e.,\,no\,surface\,effects)}}{(\Gamma)_{solution\,(i.e.,\,no\,surface\,effects)}} \times 100\% \quad (48)$$

where $(D/V)_{PRC}$ represents the percent relative change in D/V ratio, which can be zero (fresh monolayer), negative (aged monolayer), or positive (multi-layer).

If $(D/V)_{PRC}$ is negative, it will tell that there is a reduction in diameter (in this case, the height or the film thickness of the protein film) as a result of surface-induced unfolding. In other words, the spreading effects (i.e., an increase in the partial molar area occupied by the adsorbing proteins as a result of surface-induced unfolding) will result in negative $(D/V)_{PRC}$ values.

On the other hand, if there is a formation of a multilayer film, then it is projected here that $(D/V)_{PRC}$ will be positive, reflecting the stacking of protein layers above one another.

Table 1 shows the evaluation of the reference D/V_P for some selected proteins. This reference D/V_P represents the surface concentration without surface or interfacial effects, as pointed out in either Eq. (47) or (48). It is worth mentioning here that the equivalent diameter, *Deq*, represents the diameter of a sphere equal in volume to the volume as calculated by the given molecular dimensions that are shown in column 4 in Table 1.

Finally, equations (23), (26), (27), (37), and (40ab) can be used in future studies to quantify the protein adsorption process whether from kinetics or thermodynamics (i.e., equilibrium) point of view.

Conclusion

The model derivation was carried out in the most general sense and later it was simplified to special cases, including equilibrium, low surface coverage, irreversible, and Langmuirian. The percent relative change, $(D/V)_{PRC}$ parameter was introduced. $(D/V)_{PRC}$ is calculated with respect to the monolayer surface concentration of protein, where the latter is given by β/α ratio. This can be used as a landmark to protein adsorption

Table 1
Evaluation of the Reference D/V_P, which can be used in Eq. (48), Equal to the Surface Concentration without Surface Effects (i.e., $\Gamma_{solution}$)

Protein	*Molecular Weight*•	*VP (m3/mol)*•	*Dimensions (Å³)*•	*Equivalent Deq (Å)*	$\Gamma_{solution} = D_{eq}/V_P$ *(mol/m²)*	$\Gamma_{solution}$ *(μg/cm²)*
α-Lactalbumin	14161	0.010323	37 × 32 × 25	38.4	3.720×10^{-7}	0.52679
Bovine Serum Albumin	66267	0.048574	140 × 38 × 38	72.8	1.499×10^{-7}	0.99334
Human Serum Albumin	69000	0.050577	115 × 40 × 40	70.6	1.396×10^{-7}	0.96324
Lysozyme	14400	0.010512	45 × 30 × 30	42.6	4.052×10^{-7}	0.58349
Myoglobulin	17800	0.013208	44 × 35 × 25	41.9	3.1723×10^{-7}	0.56467
Ribonuclease A	13680	0.009617	38 × 28 × 22	35.5	3.6914×10^{-7}	0.50498

• Data were taken from Al-Malah (1993).

isotherms or even kinetics. This is visualized as an indicator for solid substrate effects on the adsorbing proteins. $(D/V)_{PRC}$ can be zero (fresh monolayer), negative (aged monolayer), or positive (multi-layer).

Nomenclature

k_a, adsorption rate constant [m/s]; C_o, the protein bulk concentration [mole/m³]; θ, the fractional surface coverage; A, the total surface area [m²] available for adsorption; k_d, desorption rate constant [s⁻¹]; Γ, the protein surface concentration [mole/m²]; N_s, the total number of adsorbing molecules; D, the diameter [m] of the sphere; D_{eq}, the equivalent diameter of a sphere-type protein molecule; n_P, the number of moles of adsorbing protein [moles]; V_P, the molar volume of adsorbing protein [m³/mole]; β, parameter defined by Eq. (13); α, parameter defined by Eq. (14); t, time [s]; Γ_{eq}, equilibrium surface concentration [mole/m²]; C_{eq}, equilibrium bulk concentration of protein [mole/m³]; θ_{eq}, the equilibrium fractional surface coverage; K_{eq}, equilibrium rate constant equal to k_a/k_d [m]; $(D/V)_{PRC}$, the percent relative change in D/V ratio defined by either Eq. (47), or (48).

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Appendix

The following transformation is made:

$$x = \frac{\beta}{\beta - \alpha\Gamma}$$

The Taylor series expansion will be carried out at $\gamma = 0$ (i.e., $x_o = 1$)

$$x_o = \frac{\beta}{\beta - \alpha\Gamma} = \frac{\beta}{\beta - \alpha \times 0} = \frac{\beta}{\beta} = 1$$

$$f(x) = f(x_o) + f'(x_o)(x - x_o) + f''(x_o)\frac{(x - x_o)^2}{2!}$$

$$ln(x) = ln(x_o) + (lnx)'|_{x=x_o}\ (x - x_o) + (lnx)''|_{x=x_o}\ \frac{(x - x_o)^2}{2!}$$

$$ln(x) = 0 + 1(x-1) - 1\frac{(x-1)^2}{2} \cong x - 1$$

$$ln\left(\frac{\beta}{\beta - \alpha\Gamma}\right) = \frac{\beta}{\beta - \alpha\Gamma} - 1 = \frac{\beta - \beta + \alpha\Gamma}{\beta - \alpha\Gamma} = \frac{\alpha\Gamma}{\beta - \alpha\Gamma}$$