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Kinetic Model for the Simulation of Hen Egg White Lysozyme Adsorption at Solid/Water Interface

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Abstract−A simulation model for adsorption kinetics of hen egg white lysozyme (HEW) adsorption to hydrophilic silica is proposed. The adsorption kinetic data were monitored by using *in-situ* ellipsometry. The model is based on an irreversible adsorption mechanism allowing two different adsorbed states. The adsorbed states were differentiated based on binding strengths resistant to the concentration gradient exerted by rinse. Molecules desorbing and remaining upon rinse were identified as loosely bound (state 1) and tightly bound (state 2) states, respectively. The adsorption rate constants were assumed to be a time-dependent nonlinear function in order to account for the change in surface properties originating from the protein layer formed on the surface. The parameters of adsorption rate constants were evaluated by using adsorption kinetic data at different protein concentrations, and the relationships between the adsorption parameters and protein concentration were established which eventually demonstrated a linear relationship. The established relations between the adsorption parameters and concentration elucidated the effect of protein concentration on adsorption to hydrophilic silica.

Key words: Protein Adsorption, Adsorbed State, Lysozyme, Ellipsometry, Kinetic Model

INTRODUCTION

Protein adsorption is the very first biological event when a foreign material is introduced to a physiological fluid. This physicochemical phenomenon has a variety of applications in biological, pharmaceutical, biomedical, and biochemical industries. Adsorption of blood proteins to artificial organs implanted in the human body plays an important role in triggering blood clotting cascade [Andrade, 1985]. In hard tissue engineering, adhesion proteins induce osteoblast attachment and proliferation at the implant surface, which is crucial for osteointegration [Thomas et al., 1997; Altankov and Groth, 1994]. Therefore, the success of a biomaterial may significantly depend on the proteins adsorbed on the surface of the material upon implantation.

The interfacial behavior of a protein is characterized by several factors, and extensive studies have been performed in terms of electrostatic interactions [Norde and Lyklema, 1978; Arai and Norde, 1990; Lee et al., 2002], hydrophobicity [Elwing et al., 1987; Malmsten, 1995; Tilton et al., 1991], and molecular properties such as mass and dimensions [Wahlgren et al., 1993], and surface types [Horbett, 1981].

Despite the significance of protein adsorption, the adsorption mechanism has not been fully understood. In order to facilitate industrial applications, it is desirable to develop an appropriate adsorption model. A number of studies have been conducted to build a model for protein adsorption. However, there has been little success for the development of a model which can be used for the simulation of protein adsorption. There have been a few major approaches to building an adsorption model: diffusion-limited model [Young et al., 1988; Lu et al., 1994; Adamczyk, 2000; Jason, 2001] and kinetically limited [Beissinger and Leonard, 1982; Wahlgren et al., 1995; Lee et al., 1999, 2000]. It has been widely accepted that adsorption during the initial period is a diffusion-limited process. However, the diffusion model may frequently ignore the adsorption events taking place into different adsorbed states. The model also may overestimate the later event. On the other hand, the kinetic model might underestimate the initial adsorption.

Hen egg white lysozyme (HEW) was used in this study since its molecular properties have been well-characterized and it has been widely used for the investigation of protein adsorption. HEW has an enzymatic function to destroy bacterial cell walls by hydrolyzing the $\beta(1\rightarrow 4)$glycosidic linkages from N-acetylmuramic acid (NAM) to N-acetylglucosamine (NAG) in the alternating NAM-NAG polysaccharide component of cell wall peptidoglycans [Imoto et al., 1972]. HEW consists of 164 amino acid residues and has a molecular weight of 18,700 [Matthews et al., 1973] with the dimensions of 54×28×24 Å [Weaver and Matthews, 1987].

In this study, we evaluate the adsorption kinetics of hen egg white lysozyme at solid-water interface by using *in-situ* ellipsometry. In order to account for the structural change of protein during the adsorption, adsorption kinetic data was analyzed with respect to a mechanism allowing two distinct adsorbed states.

MATERIALS AND METHODS

1. Protein and Buffer

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Hen egg white lysozyme was purchased from Sigma (L-6876) and used without further treatment. 0.01 M phosphate buffer (pH 7.2) was prepared and used in all the experiments.

2. Surface Preparation

A silicon wafer (Wacker Siltronic Corporation, OR) was used as a model surface. The wafer was cut into rectangular pieces, 1 cm× 3 cm. About 300 Å thick oxide layer was deposited on the surface by baking in furnace for 18 minutes at 1,000 °C. Then each surface was treated to be hydrophilic following the procedure previously described by McGuire et al. [1995]. All the surfaces after treatment were stored in a 50% ethanol solution in order to maintain the hydrophilicity of the surfaces. For measurement, the surface was rinsed with an excess amount of distilled deionized water and blown dry with N_2 gas.

3. Ellipsometric Measurement of Adsorption Kinetics

The surface density was measured with an in-situ ellipsometry (model L-104SA, Gaertner Corp., Chicago, IL). Initially the quartz cuvette was filled with 4.5 ml of 0.01 M phosphate buffer (pH 7.2) with a magnetic stir bar. The surface was submersed into the cuvette to be equilibrated with the buffer for at least 30 min. When the optical readings of the bare surface became steady, 0.5 mL of the two-fold concentrated protein solution was injected into the cuvette and stirred with magnetic stir bar to achieve the desired final concentration promptly. The surface was allowed to contact with protein for one hour followed by five minutes rinse with 200 ml of 0.01 M phosphate buffer. Then the surface was monitored for an additional 10 min. The change of the surface properties due to the formation of protein layer on the silica surface was measured in terms of the changes in the optical angles, ψ, and Δ. The change of the optical angles was analyzed to calculate the thickness of the adsorbed layer and converted into surface density by using the ratio of molecular weight to molar refractivity, M/A, and specific volume, ν. This computation was performed with a FORTRAN program composed according to Cuypers et al. [1983]. The values of M/A and ν of HEW were 4.19 and 0.712, respectively. Protein concentrations used were at 0.05, 0.1, 0.5, 1.0 and 2.0 mg/mL. All measurements were collected at room temperature.

THEORETICAL BACKGROUND

As indicated in the early part of this work, a number of adsorption models have been proposed to simulate protein adsorption kinetics based on diffusion or kinetics. Lee et al. [1999] proposed a model describing the protein adsorption mechanism as an irreversible adsorption into two conformational states. In the report, adsorption rate constants were given as a nonlinear function of time in order to analyze the adsorption of T4 lysozyme variants. Incorporation of time-dependent adsorption rate constants into the model, adsorption kinetics, was accurately predicted. The proposed model based on surface area occupied by protein molecules, however, has a limitation to its application when the surface density becomes greater than monolayer coverage, which can be observed when protein concentration is very high. Therefore, it is desirable to establish a model which can be utilized for a wide range of protein concentration.

For modeling purposes, the adsorbed states were differentiated into two adsorbed states according to the binding strengths characterized by resistance to rinse. Molecules in state 1 were considered to have a loose binding to the surface so that the molecules in state 1 were readily desorbed by concentration gradient generated upon rinse. Molecules in state 2 were considered to have formed a binding tight enough to overcome the concentration gradient. The model equations were expressed in terms of fraction of the surface density of each state as follows:

$$\frac{d\theta_1}{dt} = k_1 C(1-\theta_1-\theta_2) \tag{1a}$$

$$\frac{d\theta_2}{dt} = k_2 C(1-\theta_1-\theta_2) \tag{1b}$$

where, θ_i is the surface density fraction of state i (i=1 or 2) and defined as the ratio of the surface density in state i to the maximum surface density, Γ_i/Γ_{max}. The value of Γ_{max} was 0.4781 μg/cm^2 estimated at the concentration of 10 mg/mL by extrapolating the adsorption isotherm. C is the protein bulk concentration, and t is the contact time. The time-dependence of adsorption rate constants was characterized with α_i and β. The expression of the time-dependence of adsorption rate constant can be given as:

$$k_i = \alpha_i t^{-\beta} \tag{2}$$

where, k_i is the adsorption rate constant of state i. α_i is the adsorption state-specific parameter for adsorbed state i and β is the constant incorporating the change in surface properties attributable to protein layer.

The combination of the Eqs. (1a) and (1b) after substituting the function for the time-dependent rate constant, Eq. (2), for adsorption rate constants yields

$$\frac{d\theta}{dt} = (\alpha_1+\alpha_2) C t^{-\beta} (1-\theta) \tag{3}$$

where, $\theta=\theta_1+\theta_2$. The analytical solution of Eq. (3) can be obtained as follows:

$$\theta = 1-\exp\left[-\frac{\alpha}{1-\beta} C t^{1-\beta}\right] \tag{4}$$

where $\alpha=\alpha_1+\alpha_2$. Eq. (4) estimates the overall surface density but not the estimation of surface density of the individual state. Hence, the adsorption kinetics occurring into two states cannot be calculated from Eq. (4). Therefore, Eq. (4) was rearranged to differentiate the effects from 1 and 2 states as follows:

$$\ln\left(\frac{1}{1-\theta}\right) = \frac{\alpha}{1-\beta} C t^{1-\beta} \tag{5}$$

The left-hand side of Eq. (5) is a converted quantity of the overall surface density fraction. The right-hand side of Eq. (5) represents the sum of the contributions originating from states 1 and 2.

RESULTS AND DISCUSSION

In this study, a simple kinetic model is proposed in order to simulate the interfacial behavior of lysozyme. The kinetic model was based on an irreversible adsorption mechanism taking place into two different adsorbed states. The adsorbed states were characterized by the binding strength to the hydrophilic silica surface.

1. Concentration-dependent HEW Adsorption

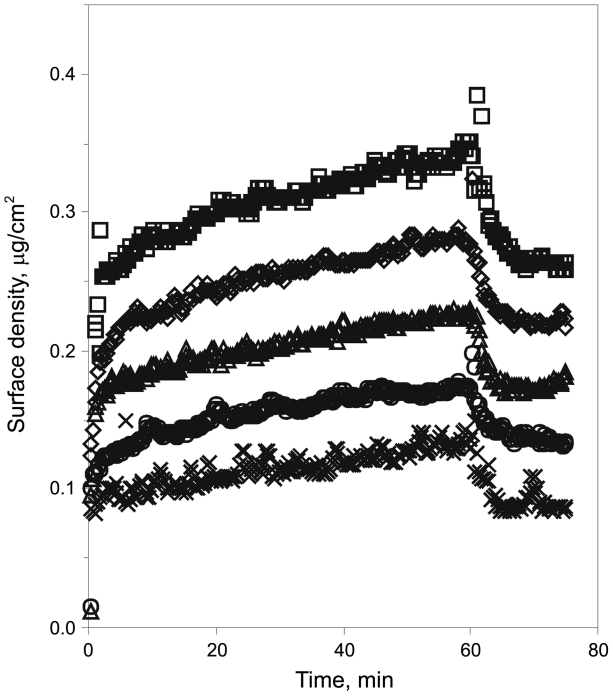

Fig. 1. Adsorption kinetic data of HEW on silica surface. The protein concentrations were 0.05 (×), 0.1 (○), 0.5 (△), 1.0 (◇), 2.0 (□) mg/mL.

Concentration-dependent HEW adsorption kinetic data to hydrophilic silica were plotted in Fig. 1. Surface density increased with incubation time, and the surface density increased with protein bulk concentration as well. The initial adsorption was extremely fast in all cases and gradually leveled off with incubation time. For HEW adsorption, Wahlgren et al. [1995] performed an experiment with HEW on hydrophilic surfaces and obtained slightly greater surface densities than measurements obtained in this study. This discrepancy might originate from the difference in surface properties.

In this study, adsorbed states were differentiated by binding strength that adsorbed molecules formed with the surface, which might be mediated by the conformational change of the lysozyme molecules upon adsorption. Tian et al. [1998] reported that the conformational change of T4 lysozyme took place upon adsorption to silica when they used circular dichroism. Therefore, tightly bound molecules might have undergone conformational change to a greater extent than loosely bound molecules in order to establish stronger binding to the surface. The resistance of the adsorbed molecules to the concentration gradient exerted by rinsing procedure was utilized for the classification of the binding strength of the adsorbed protein. HEW molecules desorbed upon rinse and which remained after rinse were considered to have formed loosely bound (state 1) and tightly bound (state 2) states, respectively. The amount desorbed upon rinse (Γ_1) and the remaining after rinse (Γ_2) were plotted in Fig. 2. The amounts in state 1, Γ_1, displayed a linear relation with respect to the HEW concentration. On the other hand, the amounts in state 2, Γ_2, appeared to be proportional to the surface densities measured at 60 min, Γ_{60}.

2. Simulation Model of Adsorption Kinetics

In order to estimate the adsorption parameters, adsorption kinetic

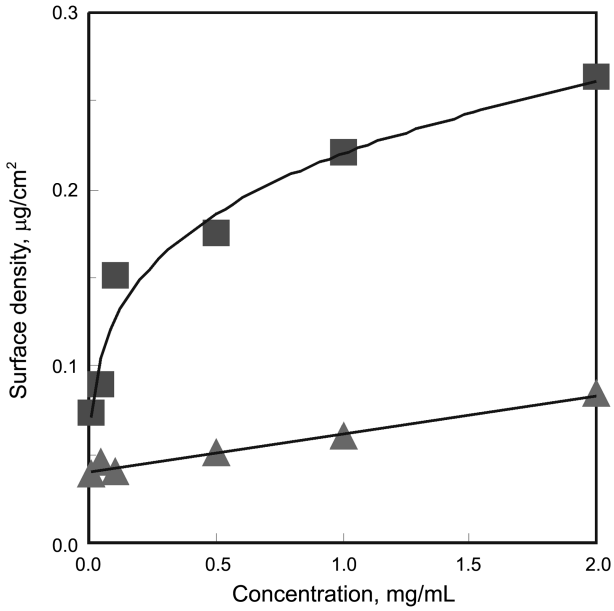

Fig. 2. Surface density of HEW on the silica surface in different adsorbed states 1 (▲) and 2 (■).

data were converted by using the expression of the left-hand side of the Eq. (5). The adsorption rate parameters, α and β, were evaluated by using MATLAB. Simulation results demonstrated the adsorption kinetic data were accurately fitted with the proposed model. Fitted curves were plotted against the converted quantities of kinetic data for all concentrations in the Fig. 3. The estimates of α and

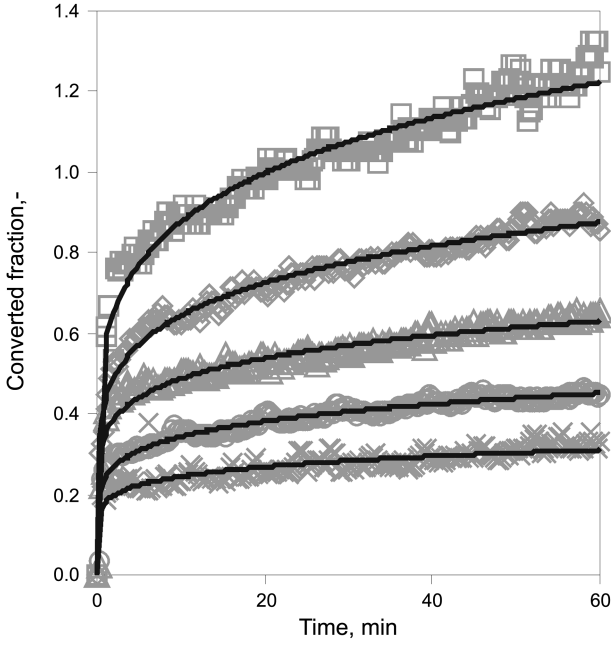

Fig. 3. Simulation results: The adsorption kinetics in the converted surface densities were fitted with proposed model. Solid curves represent the fitted results. Symbols represent the corresponding protein concentration: □ (2 mg/mL), ◇ (1 mg/mL), △ (0.5 mg/mL), ○ (0.1 mg/mL), × (0.05 mg/mL).

Table 1. Evaluated adsorption parameters, α and β, with respect to protein bulk concentrations

Parameters	Concentration (mg/mL)				
	0.05	0.1	0.5	1.0	2.0
α	0.4814	0.3684	0.1007	0.0743	0.0531
β	0.8659	0.8475	0.8558	0.8287	0.8142

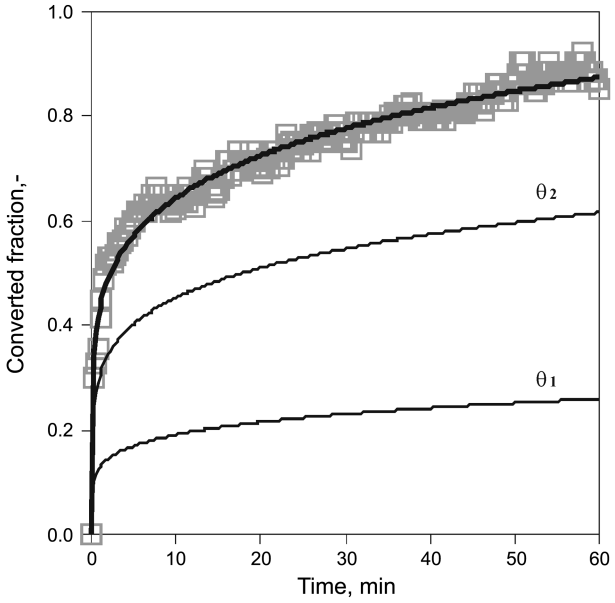

Fig. 4. Representative result of fitting the adsorption kinetics with the model allowing two adsorbed states. The protein concentration was 1 mg/mL in this case. Adsorbed states were labeled in the figure.

β were tabulated in the Table 1. The α values displayed an asymptotic decrease with the protein concentration. The β value varied but showed rather linear relationship with respect to the protein concentration. The α is the sum of α_1 and α_2 which can be calculated the experimental data of adsorbed states 1 and 2 together with the Eq. (5). A representative simulation result for HEW adsorption at 1 mg/mL is plotted in the Fig. 4 displaying the predictions of the state-wise and total adsorptions. At the same time, the predictions of the surface densities of the state 1 and 2 were also in a good agreement with the experimental measurements. The adsorption parameters for states 1 and 2 were obtained in the form of the product of parameter and bulk concentration, i.e., α_iC. The α_1C and α_2C values versus protein concentration, C, for adsorption into state 1 and 2 were plotted in Fig. 5a and 5b, respectively. The plots elucidated the linear relation between α_iC and bulk concentration, C. The mathematical expressions of the protein concentration-dependence of adsorption parameters, α_1 and α_2, are given in Eqs. (6a) and (6b), respectively:

$$\alpha_1=0.0147+0.007/C \quad (6a)$$

$$\alpha_2=0.0253+0.022/C \quad (6b)$$

The goodness of the fit was confirmed by the coefficient of determination, r^2, of the Eq. (6a) and (6b) which was greater than 0.99 and 0.95, respectively.

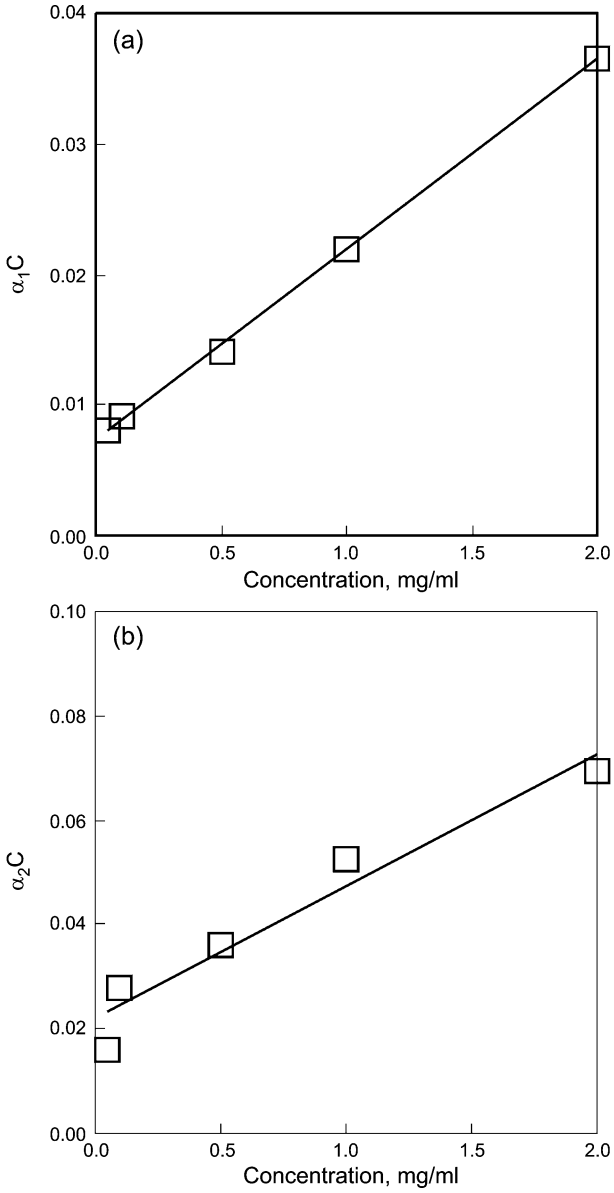

Fig. 5. The plots for the relation between the α_iC and the protein concentrations: (a) α_1C vs protein concentration, (b) α_2C vs protein concentration.

SUMMARY

Although extensive research has been devoted to the establishment of simulation models for protein adsorption, it has not been so complete to develop a model with which protein adsorption can be fully described. Furthermore, the attempts to simulate the adsorption taking place into different adsorbed states have been rarely reported and the obtained results were seldom compared with experimental observations. Even though the numerical analysis yielded optimized estimations of the parameters, the estimates might not reflect the practical events unless the estimates were confirmed with experimental observations. In this study, we proposed an adsorption kinetic model based on an irreversible adsorption mechanism

that allows two different adsorbed states based on resistance to concentration gradient exerted by rinsing with buffer. The two different adsorbed states were identified as loosely and tightly bound states. The proposed adsorption kinetic model incorporated with time-dependent rate constants successfully predicted the adsorption kinetics of HEW to hydrophilic silica for a wide range of protein concentration. The proposed model can be readily utilized in different types of surfaces and proteins, and it is expected that the concept of the proposed model can be readily applied to different types of both surfaces and proteins.

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