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The effects of denaturants on protein conformation and behavior at air/solution interface

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Abstract

In this study, we discuss the interfacial behavior of five proteins with different conformational character, and each is investigated in native and denatured states. The protein molecules are layered and spread onto the air/solution interfaces to form protein monolayer. The surface pressure–time ($\Pi(t)$) and surface pressure–area per molecule (Π–A) isotherms were measured by using the Langmuir–Blodgett (LB) balance consisted of a Nima trough system. The differences between monolayered protein's behaviors at air/solution interface indicate that denaturants, such as urea, guanidinium chloride and dithiothreitol, have different effects on conformational changes of proteins. Additionally, the interfacial behavior of the proteins in our study provides a fundamental profile about the protein structural stability and implies industrial applications in protein refolding process.

Keywords: Protein monolayer; Conformation; Air/solution interface; Denaturants

1. Introduction

Proteins are known to adsorb spontaneously to air/solution interfaces due to their amphiphilic nature [1,2]. This phenomenon is widely encountered in protein science and has been used in food and pharmacology industrial applications such as emulsions, foams and separation [3–8].

However, for the understanding in the structural character and stability of protein molecules, the protein monolayer at air/solution interface is an important issue [5,9–11]. While the protein molecules adsorb onto and then form a monolayer at air/solution interface, the free energy of system would increase and an energy barrier forms [12]. A structural rearrangement and conformational regulation of protein molecules would enhance surface pressure (as to suppress surface tension) to overcome or balance the energy barrier [13–15]. Because of the interfacial interactions, the cohesive interactions within protein molecule, such as hydrophobic and van der Waal's interactions, are interfered and disrupted [16,17]. Upon adsorption onto an interface, protein molecules tend to expand and unfold its intrinsic active structures [18,19]. Recently, a lot of observations and discussions have been reported about protein behavior at air/solution interface [5,6,8,14,19–24]. It is believed that a protein, containing higher non-polar and relatively random structures (as β-casein), favors to absorb onto and expands its conformation in a large-scale level at the interface [19,25]. The kinetic adsorption process and monolayer structural rearrangement are highly related to the polar–non-polar character of protein molecules, which relies on the consistence of amino acid residues and conformational properties, such as α-helix, β-sheet and random coil [21,22].

In this study, we demonstrate how to use the measurement of surface pressure (Π) to investigate the interfacial behavior of conformational various proteins under the effect of different chemical denaturants and elucidate the role of protein conformation in adsorption procedures and behaviors of protein molecule at air/solution interfaces.

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2. Materials and experimental methods

2.1. Materials

2.1.1. Chemicals

Five proteins with different structural characters: Lysozyme (from hen egg white, MW = 14 kDa), RNase A (from bovine pancreas, MW = 13.7 kDa), Myoglobin (from horse heart, MW = 18 kDa), β-Casein (from bovine milk, MW = 24 kDa) and Fibrinogen (from bovine, MW = 340 kDa) were purchased from Sigma (USA). Urea, guanidine hydrochloride (GdmCl) and dithiothreitol (DTT) were purchased from Sigma (USA). All chemicals in this study were ultra-pure or of analytical grade and used without further purification. Phosphate buffer (10 mM PBS, pH = 7.4, 0.09% NaCl) was prepared from highly purified de-ionized water (by Milli-Q system) and used for preparation of the protein and subphase solutions.

2.1.2. Apparatus

In this study, a computerized Nima LB trough (Nima Technology Ltd., England) uses two mobile barriers to compress the molecules and a Wilhelmy balance to measure the lateral pressure. The trough and the barrier are made of Teflon. In order to minimize evaporation and impurity from ambient environment, the trough was placed in a cabinet and temperature was kept constant at 25 °C with the aid of a water circulator bath.

2.2. Methods

The Nima trough was filled with a buffer solution without any protein. The air/solution interface was carefully aspired to remove surface impurities and the surface press was adjusted to zero. Then, a quiescent solution of protein, as the same volume as the buffer, was placed into the trough. For the study of protein adsorption behavior, the evolution of surface pressure was recorded versus time until a constant value was obtained. The surface pressure, $\Pi = \gamma_0 - \gamma$, where γ is the measured surface tension of protein solution, γ_0 the surface tension of protein solution. The $\Pi(t)$ isotherms were plotted for various proteins in different concentration (0.005–1.0 mg/ml).

Protein monolayers were spread according to Trurnits method [11] at the air/solution interface. A 25 μl aliquot of protein solution (2 mg/ml) was dripped from the top of a glass rod positioned above the air–water interface so that the solution spread uniformly on the top of the interface. After 1 h incubation of protein in various denatured solutions to unfold its conformation, the denatured protein solution was spread at air/solution interface. The surface pressure was recorded versus time and allowed to equilibrate for 1 h.

While the surface pressure reached equilibrium and the spread monolayer was then compressed by moving the two Teflon barriers (from 90 to 15 cm^2) to obtain the Π–A isotherm. All experiments were carried out in dust-free environment at 25 °C.

Table 1
The equilibrium surface pressures in different concentrations of the bulk protein solutions at 25 °C

Concentration (mg/ml)	Surface pressure, Π (mN/m)				
	Lysozyme	RNase A	Myoglobin	Fibrinogen	β-Casein
1	24.5	16.0	21.0	25.0	24.0
0.5	19.5	13.4	19.0	22.5	24.0
0.1	16.5	12.0	17.0	20.0	22.0
0.05	18.5	8.7	15.5	19.0	22.5
0.01	13.2	4.0	16.5	18.0	18.0
0.005	9.0	0	15.0	16.0	19.5

3. Results and discussion

3.1. The concentration effect of protein adsorbed onto the air/solution interface

At 25 °C, the surface pressure of bulk protein solution was measured versus time and obtained maximum equilibrium surface pressure in different concentrations and various proteins. The values of equilibrium surface pressure of protein solution in different concentrations were shown in Table 1. We could find that the higher the concentration of protein solution, the higher the equilibrium surface pressure would be. According to the Gibbs' adsorption equation, surface concentration of protein $\Gamma = -(1/RT) \times (\partial\gamma/\partial \ln C)$, where γ and C were surface tension and bulk concentration of protein solution, respectively. We know that a strong relationship existed between the Π and C, and protein adsorption onto interface is a thermodynamically favorable process [26]. However, the equilibrium surface pressure of RNase A in high concentration (1 mg/ml) is lower than that of other proteins in the same concentration. This result indicated that RNase A had the lowest surface activity and non-polar character among other proteins in this study.

The time evolution of surface pressure versus of Lysozyme in different bulk concentrations is shown in Fig. 1. The increase rates of surface pressure ($d\Pi/dt$) were higher in higher protein concentration, especially at the initial period of adsorption. And the $\Pi(t)$ curve of protein adsorption isotherm could be discriminated into two kinetic regimes. Initially, the surface pressure versus time rose sharply (first regime) and then slowed down until surface pressure reached a plateau (secondary regime). Graham and Philips [27,28] observed the similar kinetic behavior of Lysozyme adsorbed onto the air/solution interface by measuring the dynamic surface pressure $\Pi(t)$ and protein interface concentration $\Gamma(t)$. In the first regime, the surface pressure and protein interface concentration of protein solution increased synchronically. Their suggestion is that the increase of surface pressure was contributed by the transportation and adsorption or penetration of protein molecules at the air/solution interface. In the secondary regime, the $\Gamma(t)$ kept at a constant value but the $\Pi(t)$ increased continually, Graham and Philips [27,29] concluded that the event in last regime was ruled by structural rearrangement