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Review

Microcalorimetric study on conformational change of denatured RNase A adsorbed onto a moderately hydrophobic surface

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ABSTRACT

The microcalorimetric method was used to measure the displacement adsorption heat of denatured (by 1.8 mol L^{-1} guanidine hydrochloride (GuHCl)) Ribonuclease A (RNase A) adsorbed onto a moderately hydrophobic surface (PEG-600) from various ammonium sulfate ($(NH_4)_2SO_4$) concentration solutions at 298 K. According to the thermodynamics of the stoichiometric displacement theory for adsorption (SDT-A) and the measured adsorption isotherms, the adsorption thermodynamic functions, ΔG, ΔS, ΔH, and their fractions were obtained. In combination with FTIR analysis, the regulation of conformational change of adsorbed denatured RNase A was found. The results showed that the moderately hydrophobic surface can provide energy to denatured protein and make it gain more ordered conformation with $(NH_4)_2SO_4$ concentration increment. The analysis of thermodynamic fractions showed that the contribution of four subprocesses associated with the displacement adsorption of RNase A was different in various $(NH_4)_2SO_4$ concentrations.

Contents

1. Introduction 1
2. Foundation [10,11] 2
3. Experimental 2
   3.1. Materials 2
   3.2. Methods 2
      3.2.1. Adsorbed amounts 2
      3.2.2. Microcalorimetric determination 3
      3.2.3. FTIR spectroscopy 3
4. Results and discussion 3
   4.1. Adsorption of RNase A 3
   4.2. Thermodynamics of SDT-A and their fractions for adsorbed RNase A 4
   4.3. FTIR analysis 5
5. Conclusions 6
   Acknowledgement 6
   References 6

1. Introduction

Any biofluid contacted with a solid surface certainly is accompanied by a process of protein adsorption. The adsorption process may be driven by different kinds of interactions: hydrophobic and electrostatic interactions in the adsorption layer, lateral protein–protein interactions and the gain of entropy upon protein unfolding [1]. The adsorption behavior of proteins onto a liquid/solid interface has been extensively studied, and several studies have been done in this field [2–6], such as lysozyme adsorption on silanization quartz, bovine serum albumin (BSA) and lysozyme adsorption on polyvinyl alcohol affinity magnetic carrier. The thermodynamic study [7–9] performed by calorimetry of protein adsorbed onto a liquid/solid interface is a reliable method to explore the mechanism of protein adsorption [10,11]. Directly measured adsorption heats combined with adsorption isotherms

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can be used to obtain the relative magnitude of the adsorption subprocesses. Recently, the stoichiometric displacement theory (SDT) [12–14] and its thermodynamics have successfully been used for various liquid/solid adsorption systems and can elucidate the subprocesses in protein adsorption [10]. It is clear that native proteins including the reported lysozyme [10] and studying RNase A in our laboratory undergo conformational loss when adsorbed onto liquid/solid interface, but how the conformational change of denatured protein upon adsorption was seldom investigated. Therefore, in this paper we focus on the conformational rearrangement of denatured RNase A which is of more significance for exploring the mechanism of protein refolding.

Reports on protein adsorption onto hydrophobic and hydrophilic surfaces from solution illuminated that conformational change of adsorbed protein occurred [2]. Interaction between protein and surface induces conformational change of the adsorbed protein. Fourier Transform infrared (FTIR) spectroscopy technology was widely applied in quantitative analysis [15], which was an effective method to quantitatively determine protein secondary structures [2,16,17]. In order to obtain detailed information about the changes of different kinds of protein secondary structures, the initial spectra of adsorbed protein were sequentially analyzed by derivatization, deconvolution [18] and curve-fitting techniques [19,20]. Amide III (1220–1330 cm^{-1}) band was usually neglected because of its relatively weak signals but no interference from water and water vapor vibration bands and more sensitive to the change of protein secondary structures. With amide III, Xie and Liu [21] obtained the quantitative analysis results of the protein secondary structures. The results were consistent with that of X-ray measurement and amide I band data. Because the samples utilized in this study were kept dry in a thermostated desiccator, the residual water vapor signals were eliminated, if any, that can be removed by subtracting the spectrum of gaseous water. Therefore, the amide I band (1600–1700 cm^{-1}) was chosen to obtain the content of protein secondary structures.

The aim of this study is to obtain the fractions of thermodynamic functions in SDT-A from directly measured displacement adsorption heats and adsorption isotherms, and combine FTIR spectra to describe the subprocesses of displacement adsorption of denatured RNase A and to further analyze conformational change for denatured RNase A adsorption onto a hydrophobic PEG-600 packings surface.

2. Foundation [10,11]

Based on the stoichiometric displacement theory for adsorption (SDT-A) in dilute solutions, the adsorption isotherms in a liquid/solid system can be expressed as a linear equation below:

$$\ln P_a = \beta_a - \left(\frac{q}{z}\right) \ln C \tag{1}$$

where P_a is the partition coefficient of solute in the two phases, which can be calculated by adsorption isotherm [22,23]. C is the equilibrium concentration in bulk phase. β_a and q/z are constants and represent net adsorption parameters of protein and net desorption parameter of solvent in SDT-A, respectively. The general Gibbs free energy ΔG in processes of displacement adsorption including both net adsorption free energy ΔG_A and net desorption free energy ΔG_D should be:

$$\Delta G = \Delta G_A + \Delta G_D \tag{2}$$

According to the thermodynamics of SDT-A, ΔG can be written as:

$$\Delta G = -RT \ln P_a \tag{3}$$

Inserting Eq. (1) into Eq. (3) and comparing Eq. (2), ΔG_A and ΔG_D can be written as:

$$\Delta G_A = -RT\beta_a \tag{4}$$

$$\Delta G_D = RT \left(\frac{q}{z}\right) \ln C \tag{5}$$

ΔG_A is a constant while ΔG_D varies with $\ln C$. Similarly

$$\Delta H = \Delta H_A + \Delta H_D = \Delta H_A + m \ln C \tag{6}$$

$$\Delta S = \Delta S_A + \Delta S_D \tag{7}$$

$$\Delta S_A = R\beta_a + \frac{\Delta H_A}{T} \tag{8}$$

$$\Delta S_D = \frac{\Delta H_D}{T} - \left(\frac{q}{z}\right) R \ln C \tag{9}$$

where m is a constant, ΔH_A can be obtained by linear plot of ΔH *vs.* $\ln C$ in Eq. (6). It is obvious that the thermodynamic fractions of net adsorption (ΔH_A, ΔS_A and ΔG_A) are all constant in a given adsorption system.

The four subprocesses of denatured protein adsorbed onto hydrophobic surface were expressed as follows: (a) protein affinity to surface (exothermic); (b) protein molecular conformational gain (exothermic); (c) dehydration between protein molecules and surface (endothermic); (d) dehydration inside the hydrated protein molecules associated with conformation gain (endothermic). The relationship between adsorption subprocesses and thermodynamic fractions of SDT-A are as follows:

$$\Delta H_A = \Delta H_a(a) + \Delta H_{mo}(b) \quad \Delta S_A = \Delta S_a(a) + \Delta S_{mo}(b)$$
$$\Delta G_A = \Delta G_a(a) + \Delta G_{mo}(b) \tag{10}$$

$$\Delta H_D = \Delta H_d(c) + \Delta H_{md}(b) \quad \Delta S_D = \Delta S_d(c) + \Delta S_{md}(d)$$
$$\Delta G_D = \Delta G_d(c) + \Delta G_{md}(b) \tag{11}$$

where the subscripts "*a*", "*mo*", "*d*" and "*md*" represent the subprocesses (a), (b), (c) and (d), respectively.

3. Experimental

3.1. Materials

RNase A (Ribonuclease A from bovine pancreas) was purchased from Sigma Co. (St. Louis, USA), and was a globular shaped (5.3 nm × 3.8 nm × 3.0 nm) [24], a molecular weight of 13.7 kDa. PEG-600 packings made of silica base-HPHIC packings (particle size, 6.5 μm; pore diameter, 30 nm; the end-group of polyethylene glycol) were obtained from the Institute of Modern Separation Science, Northwest University, China. Guanidine hydrochloride (GuHCl) was bought from Shanghai State-medicine Group Chemical Reagent Ltd., Co. Ammonium sulfate ($(NH_4)_2SO_4$) was provided by Tianjin Nankai Chemical Reagent Co., and potassium phosphate monobasic (KH_2PO_4) was purchased from Tianjin Dengfeng Chemical Reagent Co. Other chemicals are all analytic grade. The deionized water was produced by a Millipore water filtering system (Millipore Co., Ltd., USA).

3.2. Methods

3.2.1. Adsorbed amounts

RNase A (from 0.1 to 1.0 mg mL^{-1}) in the solutions (0.05 mol L^{-1} KH_2PO_4, various $(NH_4)_2SO_4$ concentrations, pH 7.0) were denatured by 1.8 mol L^{-1} GuHCl for 24 h at 298 K. Make mixing 0.120 g PEG-600 packings with 3.0 mL RNase A solution into a batch vessel and keep shaking the mixture in an isothermal vibrator (SHA-C/TXH-82) for 3 h at 298 K until adsorption equilibrium.