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Insights into cyclodextrin-modulated interactions between protein and surfactant at specific and nonspecific binding stages

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ABSTRACT

Cyclodextrin (CD) modulated interactions between ionic surfactants with opposite charge and bovine serum albumin (BSA) at specific and nonspecific binding stages have been studied by isothermal titration calorimetry (ITC), fluorescence spectra and circular dichroism spectral measurements. At the specific binding stage with high affinity, the effectiveness of both α- and β-CD for hindering BSA–SDS interactions is quite weak; however, CD is more effective in hindering BSA–CTAB specific interactions. This is due to the cooperative electrostatic and hydrophobic interaction between BSA and SDS, and to the absence of the cooperative interaction between BSA and CTAB at the specific binding stage. For both BSA–SDS and BSA–CTAB systems (especially in the former system), α-CD is more effective in hindering BSA–surfactant interactions than β-CD. At the nonspecific binding stage, the addition of both α- and β-CD can hinder totally both BSA–SDS and BSA–CTAB hydrophobic interactions. This is caused by the more specific hydrophobic interaction between CD and surfactant compared with the hydrophobic interaction between BSA and surfactant. Our results show that the CD effect on the protein–surfactant interaction depends on both the nature of the protein–surfactant interaction and the complexing ability of CD with surfactant.

1. Introduction

Proteins and surfactants coexist in many areas such as cosmetics, foods, pharmaceuticals, biotechnology and physiological system. As a result, physicochemical interactions between surfactants and proteins have attracted considerable interest in the past years [1–6]. Both the conformation and the biological function of a protein can be modulated by surfactants [7,8]. Therefore, the ability to control the protein–surfactant interaction can be important in applications involving protein–surfactant systems. In practice, it may be desirable to first form a protein–surfactant complex to obtain some special function of protein, and then the renaturation of the protein is expected. But the removal of the surfactant from a formulation that contains numerous other compounds is not practical. In this case, an easier approach would be to "deactivate" the surfactant by adding to the solution an appropriate molecule which can bind to the surfactant and prevent it from contributing to the protein–surfactant complex formation. Cyclodextrins are molecules that can achieve such binding. Their relatively non-polar cavities are capable of accommodating a variety of molecules to form inclusion complexes [9–11]. Thus, this ability of CD can be exploited in modulating the properties and functionality of protein–surfactant complexes. Despite the significance of such systems, the interaction mechanism of cyclodextrins and mixtures of protein and surfactants remain unclear [12].

Native BSA is a globular protein with second structure consisting of hydrogen bonded α-helices (62%) and β-sheets (7%) [13,14]. The proposed structure of bovine serum albumin (BSA) is heart shaped, consisting of three homologous domains (I, II, III). Each domain is composed of two subdomains. The subdomains share a number of common features, namely, a hydrophobic face and a cluster of basic amino acid residues. It is established that each subdomain is unique and exhibits a certain degree of binding specificity. Protein denaturation is often followed by a massive "unfolding" of the protein, and undergoes structural changes and transforms into its unfolded configuration with the loss of second and tertiary structure. The interaction of BSA with the anionic surfactant sodium dodecylsulfate (SDS) as well as the cationic surfactant cetyltrimethylammonium bromide (CTAB) has been extensively studied aiming to understand how surfactant binding affects the protein structure and function. For both SDS [15–21] and CTAB [18,22–24], surfactants bind to BSA via two typical binding stages, i.e. specific and nonspecific stage. The specific binding region, at very low surfactant concentration, is associated with the binding of surfactant monomers to the specific sites of the proteins and the interactions are combinations of electrostatic and hydrophobic interactions. At the nonspecific binding stage, with the higher surfactant concentration, surfactants bind to protein mainly via hydrophobic interaction.

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Based on these results, we investigate the effects of α- and β-cyclodextrin on the interaction between BSA and surfactant with linear hydrophobic tail at specific and nonspecific binding stages (especially in the former stage) in the present work. Different from earlier studies that cyclodextrin inhibits effectively the nonspecific interaction between protein and surfactant with linear hydrophobic tail, regardless of the CD type [25], some interesting and significative results have been gained. It is found that the effect of cyclodextrin on the protein–surfactant specific interaction depends on both the nature of the protein–surfactant interaction and the association constant between CD and surfactant. The present work provides new insights into the effect of cyclodextrin on the interaction between protein and surfactant at the specific binding stage, which contributes to understanding the effect of cyclodextrin on the properties and functionality of protein–surfactant complexes. This will in turn facilitate the use of these materials in foods, pharmaceuticals, biotechnology and related industries.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA), sodium dodecylsulfate (SDS) (99% purity) and cetyltrimethylammonium bromide (CTAB) (99% purity) were supplied by Sigma and used without further purification. The purity of surfactant was tested using surface tension measurements and no minimum in the surface tension–surfactant concentration profile was observed. Both α- and β-cyclodextrin were purchased from Aldrich and used without further purification. All samples were prepared in 5.0 mM sodium phosphate buffer at pH 6.8. The BSA concentration was kept at 0.01 mM. All other reagents used were of analytical grade from Shanghai Chemical Reagent Company, and ultrapure Millipore water (18.2 MΩ cm) was used as the solvent.

2.2. Microcalorimetry

Heats of titration were measured using a VP-ITC titration microcalorimeter from MicroCal Inc., Northampton, MA at 298 K following standard instrumental procedures with a 250-μL injection syringe and a 1.438 cm^3 sample cell. The duration of each injection was 10 s, and the time delay (to allow equilibration) between successive injections was 240 s. The contents of the sample cell were stirred throughout the experiment at 307 rpm to ensure thorough mixing. Raw data were obtained as a plot of heating rate (μcal s^{-1}) against time (min). These raw data were then integrated to obtain a plot of observed enthalpy change per mole of injected SDS or CD (ΔH_{obs}, kcal mol^{-1}) against SDS concentration (mM) or C_{CD}/C_{SDS}. Integrated heat pulse data, corrected for heats of mixing and dilution, were analyzed by nonlinear regression methods in terms of standard equilibrium binding models, using Microcal ORIGIN software, to give estimates of equilibrium association constant, and enthalpy of complex formation. All experiments were repeated at least twice, and the reproducibility was within ±2%.

2.3. Steady-state fluorescence

Steady-state fluorescence experiments were performed with a RF-5301 luminescence spectrometer (Japan Shimadzu Company) equipped with a thermostated water-circulating bath. During the experiments, for BSA solution, the excitation and emission slits were fixed at 3.0 and 1.5 nm, respectively. The excitation wavelength was set at 295 nm, and the emission spectra were collected from 300 to 500 nm.

2.4. Circular dichroism

Circular dichroism spectra were recorded on a JASCO J-815 spectrophotometer at 298 K, using a 1.0-cm quartz cell. Scans were obtained in a range between 190 and 360 nm by taking points at 0.1 nm, with an integration time of 0.5 s. Typically, three spectra were averaged to improve the signal-to-noise ratio.

3. Results and discussion

3.1. Effect of CD on the interaction between BSA and SDS

It must be mentioned firstly, ITC and fluorescence results show that the addition of α- and β-CD to 0.01 mM BSA aqueous surfactant–free solution does not affect the structure of BSA (Fig. S1). This implies no interaction between CD and BSA, which is consistent with the previous results [26]. Thus, the effect of CD on BSA and SDS interaction are mainly caused by surfactant and CD interactions.

3.1.1. Interaction between BSA and SDS in the presence of CD

ITC is one of the most sensitive techniques that permit the direct measurement of thermodynamic changes in the course of binding of surfactant to protein [17,27]. Fig. 1A and B shows the ITC curves of titrating 0.5 mM SDS in BSA, CD and BSA–CD mixture solutions. As shown in Fig. 1A, in the absence of CD, the binding of SDS to BSA is exothermic and the two classes of binding sites can be clearly resolved. At the lowest concentration, SDS molecules bind to specific high affinity sites on the protein by hydrophobic interaction modulated by electrostatic nature. At 0.05 mM SDS (c'), these high affinity sites are fully saturated and the continuous binding gives rise to the second plateau of the titration curve. These low affinity sites appear saturated when the SDS concentration exceeds 0.12 mM.

It can also be seen from Fig. 1A, at $C_{SDS} < 0.05$ mM, the presence of β-CD hardly affect the observed enthalpy change of SDS titrating BSA solution alone, but the deviation between the two titration curves with and without β-CD becomes more obvious with the increase of C_{SDS} at $C_{SDS} > 0.05$ mM. Finally, the curve of titrating SDS in BSA/β-CD mixture solution approaches to the curve of titrating SDS in β-CD solution alone. The results here indicate that the specific interaction between SDS and BSA with high affinity is hardly inhibited in the presence of β-CD. However, the interaction between the non-polar cavity of β-CD and the hydrophobic chain of SDS can hinder the specific interaction between SDS and BSA with low affinity to some extent. Differently, as shown in Fig. 2A, ΔH_{obs} of SDS titrating BSA–α-CD mixture solution deviates much from that titrating BSA alone solution even at the lowest SDS concentration. This means that specific interactions between SDS and BSA with high and low affinity have been both hindered effectively by α-CD. Thus, α-CD is more effective than β-CD in modulating the specific interaction between SDS and BSA, especially at lower SDS concentration, where SDS molecules bind to BSA via specific interaction with high affinity.

As is well known, cyclodextrins are cyclic oligosaccharides in the shape of truncated cones made up of several units of α-glucopyranose linked by α-1,4 glucoside bonds. Their relatively non-polar cavities are capable of accommodating a variety of molecules (including surfactants) to form inclusion complexes [9–11]. Thus, cyclodextrin present in the system has the potential to control the BSA–SDS interaction. For α-CD, the internal diameter and depth are approximately 5 and 8 Å, respectively, whereas these parameters have values of 7 and 8 Å, respectively for β-CD. The cavity size of α-CD is smaller than that of β-CD, so the inclusion of a linear hydrocarbon chain in the α-CD cavity results in a tighter fit, hence a larger binding constant. This is further confirmed by the