Colloids and Surfaces A: Physicochem. Eng. Aspects 483 (2015) 36–44

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

journal homepage: www.elsevier.com/locate/colsurfa

Investigation of bovine serum albumin-surfactant aggregation and its physicochemical characteristics

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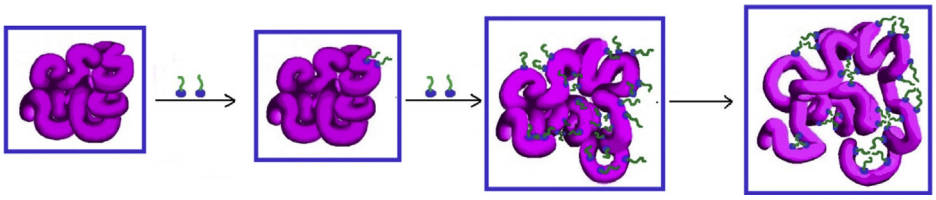
HIGHLIGHTS

- Synergistic interaction between surfactant and BSA has been suggested from surface tensiometry and fluorimetry data.
- Fluorimetry study and DSC study indicate the appearance of more nonpolar regimes at the BSA–water interface.
- The mechanism of formation of a BSA-surfactant mixed aggregate has been suggested.
- The surfactant hydrocarbon chain length is shown to play vital role in protein unfolding.

GRAPHICAL ABSTRACT



Schematic representation of surfactant induced unfolding of Bovine serum albumin in aqueous solution.

ARTICLE INFO

Article history:
Received 17 March 2015
Received in revised form 24 June 2015
Accepted 30 June 2015
Available online 4 July 2015

Keywords:
Protein–surfactant interaction
Zeta potential
Dynamic light scattering
Aggregation number
Differential scanning calorimetry
Protein denaturation

ABSTRACT

The mechanism of the formation of aggregates between a protein, bovine serum albumin (BSA), and alkyltrimethylammonium bromides of varied hydrocarbon chain lengths, namely, cetyltrimethylammonium bromide (CTAB), tetradecyltrimethylammonium bromide (TTAB), and dodecyltrimethylammonium bromide (DTAB) in an aqueous solution and the physicochemical characteristics of the aggregates were systematically investigated by surface tensiometry, fluorimetry, UV–vis spectrometry, dynamic light scattering, zeta potential, and differential scanning calorimetry (DSC). The surface tension and fluorimetry data indicate a steady decrease in the critical micelle concentrations of the surfactants with an increase in the amount of BSA in the mixture. The evolution of an additional nonpolar segment in the backbone of BSA was indicated by the fluorescence of pyrene and the intrinsic fluorescence of BSA as well. The decrease in the aggregation number, increase in the area per molecule of the surfactant at the interface with a concomitant increase in the hydrodynamic radius of the aggregate were attributed to the formation of BSA–surfactant mixed aggregate and the induction of the unfolding of BSA by the surfactants. The DSC study and nature of the denaturation curves of BSA indicate that the stability of the BSA–surfactant complex follows the order: CTAB > TTAB > DTAB. The neutralization of the negatively charged surface of BSA by the positively charged surfactants is evident from the zeta potential measurements. Both the head group and nonpolar moiety of the surfactants affected the surface charge of the aggregates and the studied surfactants denature and unfold BSA; the extent of denaturation is predominately decided by the hydrocarbon chain length of the former. The in situ unfolding of the protein and the subsequent formation of the aggregates are proposed. The characteristic parameters of the aggregates were determined.

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http://dx.doi.org/10.1016/j.colsurfa.2015.06.052
0927-7757/

1. Introduction

Proteins are one of the primary components of the living cells that account for the growth and activities of the biological systems. Proteins are turned and twisted in a definite spatial arrangement to organize themselves to minimize the free energy constraints due to the electrostatic, hydrogen-bonding, van der Waals, and hydrophobic interactions among their amino acid residues and with the surrounding water molecules [1]. Consequently, a large fraction of the hydrophobic side chains is buried in the interior of the molecules in the most stable conformational state [2]. The protein defolding, however, exposes some of the hydrophobic moieties of protein to water [3,4]. In such altered conformational states, protein molecules can readily interact with nonpolar substrates [5]. The interactions of proteins with other molecules, particularly surfactants, are of significant importance not only because of their fascinating structural organization, but also because of their potential technological applications in industry, biology, pharmaceutical, and personal care products and therefore, attracted a great deal of interest for many years [6]. Although the importance of the interaction between proteins and surfactants has been realized since long, nonetheless the fundamental understanding of the molecular mechanisms underlying both the protein–surfactant interactions and role of these interactions in various applications are still moderately understood [7,8]. Mostly, electrostatic interaction and hydrophobic association are the two main driving forces that account for the protein–surfactant interactions [9,10].However, the comprehensive understanding of the mechanism of the organization, size, and micropolarity of the protein–surfactant organized assemblies and the nature of the interfaces are crucial parameters that need to be investigated in addition to the prevailing interactions to orient the protein–surfactant interactions suitable for desired applications.

Verdes et al. [11] studied the thermodynamics of the micellization of different surfactants, e.g., sodium dodecyl sulfate (SDS), sodium octanoate, and sodium perfluorooctanoate in the presence of the protein, human serum albumin (HSA), in water. The critical micelle concentrations (CMCs) of the surfactants changed due to the interaction with protein. Moreover, at lower concentrations, the adsorption of surfactants on the protein surface protected the thermal unfolding of HSA. SDS showed a higher protective effect than sodium octanoate. From the studies on the binding of the cationic gemini surfactant, alkanediyl-α,ω-bis-(dodecyldimethylammonium bromide) ($C_{12}C_SC_{12}$-Br) and single-chained surfactant, dodecyltrimethylammonium bromide with bovine serum albumin (BSA), Lei et al. [12] found out that the gemini surfactants have a stronger ability to bind to the protein. They attributed this phenomenon to the presence of two charged centers and larger hydrophobic regions in gemini surfactants, and therefore, the latter denatured BSA appreciably than the single-chained surfactants. The study on the micellization of the cationic surfactant, dodecyldimethylethylammonium bromide (DDAB), in the presence of BSA [13] revealed that the conformation of BSA was affected at a lower concentration of the surfactant. However, at higher concentrations (>CMC), DDAB did not interact with BSA due to the micelle formation. The cationic surfactant changed the interaction propensity of protein with the drug, thus decreasing the drug side-effect [14]. Protein–surfactant interactions involving both ionic and nonionic surfactants have been studied [15]. The surfactant-induced conformational changes of BSA have been confirmed by time-resolved fluorescence and circular dichrosim studies. However, among the three categories of surfactants, nonionic surfactants showed the least interaction with BSA. The interaction of cetyltrimethylammonium bromide (CTAB) with BSA has been studied by small-angle neutron scattering (SANS), fluorescence, and circular dichroism [16]. At a low [CTAB], the protein shows a native-like behavior. However, at a high [CTAB], a 'necklace model' of micelle-like clusters randomly distributed along the polypeptide chain has been observed [16,17]. The overall size of the complex increases with increasing surfactant concentration.

The conformational changes in the BSA–SDS complexes and the size of the micelle-like clusters distributed along the polypeptide chain have been evidenced from surface tension technique coupled to small-angle X-ray scattering (SAXS) measurements [17]. The results indicate that the detergent did not modify the native protein conformation. However, the beginning of protein unfolding is coincident with the onset of SDS cooperative binding to BSA. Despite a large number of reports relating to BSA–surfactant interactions, the nature of protein–surfactant interaction at the molecular level has been rarely studied. Also, many other characteristic parameters of the mixture such as the size, micropolarity of the aggregates, and orientation of the surfactants at BSA–water interface, which are indispensable for predicting and controlling further applications, have not been studied.

Our primary interest has been centered on the studies of the interactions prevailing within the single or mixed systems involving surfactants and the organization/orientation of the surfactants [18–22] within the organized assemblies. Herein, we analyzed the mixtures of BSA with CTAB (C_{16}), tetradecyltrimethylammonium bromide (TTAB, C_{14}), and dodecyltrimethylammonium bromide (DTAB, C_{12}) in aqueous solutions to investigate largely the physicochemical characteristics of the mixtures and the mechanism of the organization of the assemblies. The surfactants with a common hydrophilic head (trimethylammonium ion) and variable hydrophobic chain lengths were selectively selected to decipher the role of the surfactant structure on the mechanism of the organization in BSA–surfactant systems. BSA is a native protein and is obtained as the by-product of the cattle industry. BSA is composed of a large number of amino acids such as tryptophan (Trp), tyrosine (Tyr), and lysine along with disulfide bonds [23]. BSA is mostly preferred because of its stability, low cost, and inactivity in many biochemical reactions. Although BSA is highly stable, the balance between folded and unfolded structures can be changed delicately by various factors such as temperature, pH, inorganic salts, organic solvents, detergents, and pressure [24]. Therefore, we assume that a systematic investigation of surfactant–BSA mixtures would be highly prospective and imperative. Our results conclusively elucidated the mechanism of the formation of the aggregates at the molecular level and different characteristic parameters of the aggregates such as their size, surface charge, aggregation number, micropolarity, and the orientation of the surfactants at the interfaces.

2. Materials

The cationic surfactants under investigation, i.e., CTAB, TTAB, and DTAB (MERCK, Germany), were recrystallized from an alcohol/acetone mixture [25] before use. The absence of the minima in the surface tension—concentration curves indicated the high purity of the sample [26]. CPC (cetyltrimethyl pyridinium chloride) and pyrene (Aldrich, USA), and BSA (Merck, Germany, molecular weight MW = 66000 D, purity >98.5%) were used as received. Triple-distilled water (conductance = 1×10^{-6} mho) was used within seven days of its preparation. Freshly prepared solutions of BSA and surfactants in phosphate buffer of pH 7.4 with triple distilled water [27] were used for each measurement. Different percentages of BSA solution were prepared by dissolving BSA in grams per 100 mL of the solution. The experiments were performed at ambient temperature of T = 303.15 K (SD = ± 0.2 K, confidence level = 0.68) and at a