



On the Interactions of Bovine Serum Albumin with Some Surfactants: New Insights from conductivity Studies.

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ABSTRACT

The interactions of bovine serum albumin (BSA) with one anionic, sodium dodecyl sulfate (SDS), cationic Surfactant cetyl trimethylammonium bromide (CTAB), and a nonionic triton X-100 (TX-100), at 25 °C in aqueous media containing phosphate buffer (20 mM, 0.1 M NaCl, pH 7.0) have been studied through the conductivity method. A novel and simple mathematical model was used for calculation of mole fractions for each surfactant interacted with BSA. The results revealed that SDS interact more strongly than CTAB and TX-100. The results indicated that the SDS, CTAB and TX-100 comprise 98%, 88% and 31% interaction with BSA, respectively. The results were interpreted on basis of hydrophobic interactions of side chain of surfactant with BSA hydrophobic surface patches on BSA.

Keywords bovine serum albumin, sodium dodecyl sulfate, dodecyl trimethylammonium bromide, triton X-100, interaction parameters, conductivity method.

INTRODUCTION

Proteins are the most important life-forms in the world which have intimate relationships with life activities such as nutrition, development, heredity and metabolism. Globular proteins are used as functional ingredients in a variety of food, health care and pharmaceutical products because of their abilities to catalyze enzyme reactions, adsorb to surfaces, bind other molecules and form molecular aggregates (1-3). Serum albumin (SA) is one of the most widely studied proteins. It is the most abundant protein in the circulatory system. Bovine serum albumin (BSA) is a single-chain protein and belong to serum albumins. The globular protein BSA in aqueous solution has been extensively studied in the past by several techniques which indicate that the defatted BSA molecule has a prolate ellipsoidal shape having a molecular weight of 66700 ± 400 Da (4-6). Bovine serum albumin (BSA) is a very interesting biophysical and biochemical system and has been quite studied over many years. Its primary structure is very well known consisting of 585 amino acid residues, whereas its secondary structure contains 67 % of alpha helix and 17 disulfide bridges that confer to the protein a remarkable stability (7).

The BSA molecule (Figure 2) is made up of three homologous domains (I, II, III) which are divided into nine loops by 17 disulphide bonds. The loops in each domain are made up of a sequence of large-small-large loops forming a triplet. Each domain in turn is the product of two subdomains (IA, IB, etc.) (8).

The interactions of proteins with surfactants provide valuable models for understanding the binding of proteins with small molecules such as hormones.

Interactions of surfactants with proteins are very important in a wide variety of industrial, biological, pharmaceutical, and cosmetic applications. Interaction of globular proteins, especially BSA with surfactants, in particular sodium dodecyl sulfate, SDS, has been extensively studied aiming to understand how surfactant binding

affects the protein structure and function (9). Several techniques have been used to investigate the assembled protein-surfactant complexes. (10-13)

Moreover, the study of the nature of the interactions between proteins and surfactants provides insight into the action of surfactants as denaturants and as solubilizing agents for membrane proteins; systems of this type serve as models for the interactions that occur between membrane proteins and lipids. The interaction between globular proteins and n-alkyl sulphates has been extensively investigated. (14 and 15).

There are several studies that are widen the range of the researches of the interactions of the protein with surfactants, but not enough to understand the role of the hydrophobic and electrostatic interactions between BSA and surfactant. The aim of the present study, was to study the interactions of BSA with some selected surfactants by the conductivity measurements. The surfactants chosen here were anionic surfactant sodium dodecyl sulfate (SDS), cationic surfactants cetyl trimethylammonium bromide (CTAB) and nonionic surfactant (Triton X-100), Figure 1. Also, the mol fractional ratios of both free and interacted surfactants and BSA separately, will be calculated in this work.

The structures of SDS, CTAB, and TX-100, and BSA are represented in figure 1.

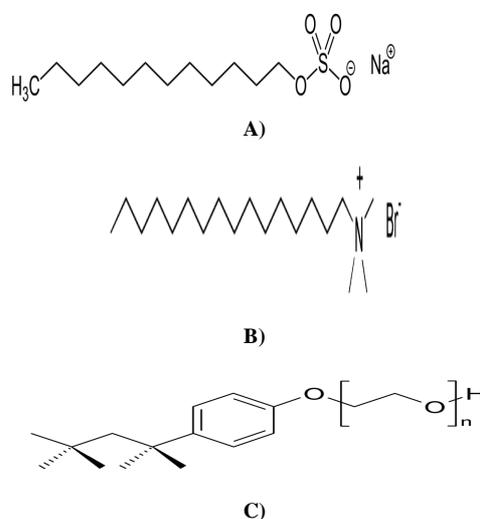


Fig. 1: Schematic representation of : Surfactants (A): Sodium dodecyl sulfate, (SDS) (B): Cetyl trimethylammonium bromide , (CTAB) and (C): Triton X-100 (TX-100).

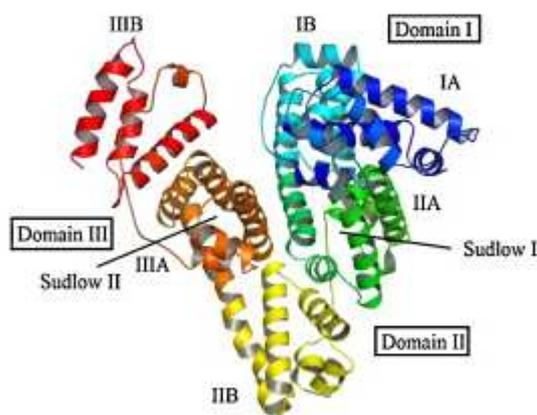


Fig. 2: A ribbon representation of modeled BSA with the three domains (I-III) and the corresponding subdomains showing the locations of domain-binding sites. The locations of hydrophobic binding sites (Sudlow I and Sudlow II) are indicated.

EXPERIMENTAL SECTION

Materials

The ionic surfactants SDS and CTAB were purchased from Alpha and Merck companies, respectively. Triton X - 100 was purchased from Oxford laboratory reagent company. BSA was obtained from Alpha company, Citric acid was obtained from HiMedia Laboratories Pvt. Ltd, and dibasic phosphate was obtained from Oxford laboratory reagent company.

Unless otherwise noted, all reagents and solvents used in this study were used as received without further purification. All solutions in this study were prepared with doubly distilled water.

The phosphate buffer was 20 mM (containing 0.1 M NaCl) and its pH was adjusted to 7.00 with HCl using a Delta 320-S acidity meter (Mettler Toledo, Shanghai, China).

Conductivity measurements

The conductivities for the studied surfactant/BSA systems were determined at 25°C by a AD3000 EC/TDS accompanied by thermal probe Conductometer (Chempal company). The instrument had been calibrated by the utilized KCl solution.

Measurements of UV-Vis absorption spectra

The BSA concentration was determined spectrophotometrically on a Thermo Fischer scientific genesys 10 UV spectrophotometer.

Buffer phosphate pH 7 was used as a blank. from absorbance measurements at 278 nm using a molar extinction coefficient of $4.4 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ at 280 nm. [16]

Stock solutions of BSA and the selected surfactants SDS, CTAB and Triton X-100 (10 mM each) were prepared by dissolving the calculated amount in a proper volume of 20 mM, (0.1 M NaCl) phosphate buffer solution pH 7.

stock surfactant solutions of 40 mM were prepared in phosphate buffer solution (20 mM, 0.1 M NaCl, pH 7.0). The BSA solution was freshly prepared in the same buffer solution, and treated at final concentration of 20 μM with different surfactant solutions of concentration range from zero up to 3 mM for each surfactant studied, and the conductance of each prepared solution were recorded after 1 min of addition .

Procedure

All BSA samples were prepared in 20 mM phosphate buffer at pH 7.0 (above its pI). The samples were made just before the measurements. The final concentration of BSA was 20 μM.

RESULTS AND DISCUSSION

Conductivities of surfactant-BSA

The interactions of bovine serum albumin (BSA) in presence of anionic (SDS), cationic (CTAB), and non ionic (TX-100) surfactants at the studied surfactant concentration (up to 3 mM) at 25 °C in 20 mM phosphate buffer solution of pH 7.0 were studied by measuring the conductivity of the surfactant/BSA solution systems. The interactions occurred can be easily detected by analyzing the conductivity profiles of the corresponding surfactant/BSA solution systems.

Figure (3) shows the conductivity profiles (κ) of the surfactant/BSA system versus the concentration of surfactant. From figure 1, it is seen that the conductance (κ) of ionic surfactants (SDS and CTAB) is decreased with the increase of the concentration of the ionic surfactants, being more steeply decreased in case of SDS. while it exhibit a reverse trend with the non ionic surfactant (triton X-100).

Conductivity profiles (Fig. 3) could be interpreted in terms of various molecular events, e.g. the ionic nature of the surfactant studied as well as the hydrophobic nature of the side chain associated with the studied surfactant.

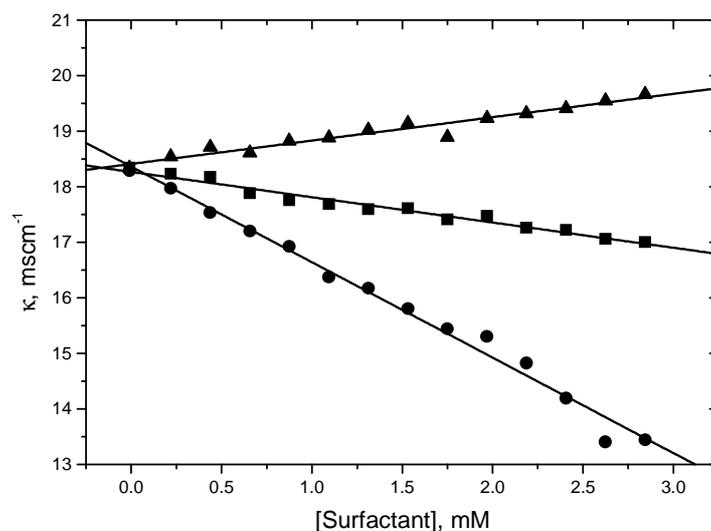


Figure 3: Conductivities of aqueous surfactant/BSA solution systems. CTAB (squares), SDS (circles), and Triton X-100 (up triangles). Under all experimental conditions, the final concentration of BSA = 20 μM in phosphate buffer (20 mM, 0.1 M NaCl) of pH 7 at 25 $^{\circ}\text{C}$.

D. Kelley and D. J. McClements (17) suggested that the interactions of bovine serum albumin (BSA) with cationic Dodecyl trimethylammonium bromide (DTAB) and anionic (SDS) surfactants in aqueous solution (pH 7.0, 20 mM imidazole, 10 mM NaCl) BSA was completely denatured when the DTAB concentration exceeded about 4 mM, and BSA became completely denatured when the SDS concentration exceeded about 4 mM.

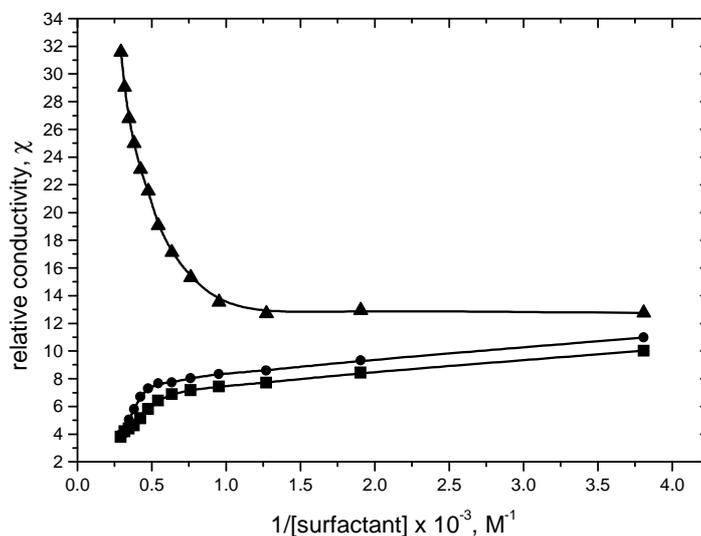


Figure (4): Double reciprocal plots of relative conductivity versus reciprocal surfactant concentration for interaction of CTAB (■); SDS (●) and TX-100 (▲) with BSA. Initial BSA concentration was 20×10^{-6} M, and the surfactant concentrations ranged in 0 – 3 mM. All experiments were performed at 25 $^{\circ}\text{C}$ in phosphate buffer solution of pH 7.0

Figure (4) shows the dependence of the relative solution conductivity (χ) of the solution systems surfactant/BSA on the reciprocal surfactant concentration. In which χ is given in equation (1)

Where, K and K_0 represent the conductivity of BSA solution in presence and absence of surfactant, respectively.

It is shown in figure (4) that the relative conductivities (χ) for the interactions of ionic surfactants (SDS and CTAB) with BSA were in an inverse proportion with the surfactant concentration in contrast with that of the nonionic (TX-100) surfactant. Moreover, the conductivity data show a small change in the relative conductivities of the three surfactants after about 2 mM surfactant concentration.

Interaction parameters

The values of K_d for the surfactant/BSA interactions were calculated according to equations 1 and 2. From the values of K_d , and the molar concentrations of the studied surfactants with BSA.

The values of the molar fractional values of surfactant interacted with BSA (W) obtained are different for the three surfactants studied and may provide some clues to the kinds of interaction processes involved. The molar fractions of the interacted and free both, surfactant and BSA, separately (i.e W, X, Y, and Z) for the interactions surfactant/BSA was calculated using a mathematical model given by equations (3 - 6) (18 - 21) in which (χ) is given in terms of the ratio of conductances in presence (κ) and absence of BSA, (κ_0).

$$\chi = \frac{1}{\left(1 - \frac{\kappa}{\kappa_0}\right)} \dots\dots (1)$$

The values of interaction constant (K_d) for the interaction surfactant/BSA were calculated according to equation similar to equation given by (22).

$$\chi = \frac{K_d}{[\text{surfac tan } t]} + 1 \dots\dots\dots (2)$$

The values of K_d for the interactions surfactant/BSA are reported in table (1)

Table (1): Interaction parameter (W) of BSA/surfactant under experimental conditions

Surfactant	K_d	W	X	Y	Z
SDS	1.8×10^{-5}	0.98	0.02	0.065	0.935
CTAB	0.000202	0.88	0.12	0.059	0.941
Triton X-100	0.00336	0.31	0.69	0.021	0.979

The increase in K_d values may be attributed to two factors, (i.e increasing hydrocarbon chain length of the surfactants and the electrostatic interaction). Therefore, the hydrophobic interactions between the hydrocarbon tail of the surfactant and hydrophobic patches on the surface of the protein play an important role in the initial interactions of the ionic surfactants, SDS and CTAB with BSA.

The molar fractional values of surfactant interacted with BSA, (W) of the molar fraction of surfactant free BSA (X), of molar fraction of BSA-interacted surfactant (Y), and of molar fraction of the BSA-free surfactant (Z) have been calculated according to a model previously used by Bocedi, et al (18).

The interaction parameters (W, X, Y, and Z) for the interaction of studied surfactant/BSA listed in table (1) were calculated using equations (3-6) :

Where:

W: mole fraction of surfactant-bounded BSA.

X: mole fraction of surfactant-free BSA.

Y: mole fraction of BSA-bounded surfactant, and

Z: the mole fraction of BSA-free surfactant.

$$W = \{[\text{surfac tan } t]/[\text{BSA}] + K_d /[\text{BSA}] + 1 - \sqrt{([\text{surfac tan } t]/[\text{BSA}] + K_d /[\text{BSA}] + 1)^2 - 4 \times [\text{surfac tan } t]/[\text{BSA}]\} / 2 \quad (3)$$

$$X = 1 - W \quad (4)$$

$$Y = W \times [\text{BSA}] / [\text{surfac tan } t] \quad (5)$$

$$Z = 1 - Y \quad (6)$$

For each surfactant, molar fraction values (W, X, Y and Z) for the interactions surfactant/BSA are reported in table (1). The data here reported support the hypothesis that the ionic surfactants are strongly interact with BSA and enhance its denaturation.

According to data depicted in table (1) it is seen that the anionic surfactant SDS has the highest value of W probably, due to interaction occurred between the negatively charged since the isoelectric point (pI) of BSA is about 4.7 (23) and the pH at which the interaction (SDS/BSA) was occur about pH7. Thus, the BSA is negatively charged and there exists a greater electrostatic interaction of SDS/BSA. which support the idea that BSA may exhibit a packed structure with SDS bounded by electrostatic interactions. The hydrophobic interactions between the hydrocarbon tail of the surfactant and hydrophobic patches on the surface of the protein play an important role in the initial interactions. (24)

The application of the previous mathematical model to the experimentally determined values (Table 1) indicates that the molar fraction (W) of studied surfactants interacted with BSA follow the order: SDS > CTAB > TX-100 which indicate that SDS, CTAB and TX-100 comprise 98%, 88% and 31% interaction with BSA, respectively.

Nozaki et al., (25) suggested that the anionic surfactants such as SDS has a stronger interaction with the protein compared to cationic surfactants.

While the non ionic surfactant (Triton X-100) results in $w = 0.31$, the ionic surfactants (SDS, and CTAB) interaction result in values of ($w = 0.98$ and $0.0.88$ respectively). This suggests different behavior in the interaction processes between these surfactants/BSA.

Based on the molar fraction values (W) in table (1), the apolar side chains in the ionic surfactants SDS and CTAB may explain the fact that both surfactants are easily interacted with the negatively charged residues in BSA at pH 7 in the hydrophobic pocket of BSA. It is obvious that the affinity of binding is increased with increasing length of the hydrocarbon tail of the surfactants. This is further evidence that the hydrophobic tail of the surfactant plays an important role in the interaction with BSA.

The results depicted in table (1) indicated that the anionic surfactant (SDS) is the most strongly interacted with BSA, while the anionic surfactant (TX-100) is the least interacted. This result supports the hypothesis that these surfactants may contribute to a certain extent in the denaturation of BSA. (26)

CONCLUSION

In this work, the interaction of three well selected surfactants (SDS, CTAB and TX-100) at concentrations (0 - 3 mM) with BSA was investigated at 25 °C through measuring the conductivities of surfactant/BSA solution systems. The experimental data indicate that a different behavior is observed for the three surfactants studied, and the extent of interaction follows the order SDS > CTAB > TX-100.

Moreover, we calculated the various molar fractions for free and bound surfactant and BSA, separately. This work give a useful guideline for such interactions surfactant/protein, and so it had a significance in methodology and understanding the protein-surfactant interaction mechanisms.

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