Interaction between bovine serum albumin and equimolarly mixed cationic–anionic surfactants decyltriethylammonium bromide–sodium decyl sulfonate

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Abstract

The interactions of bovine serum albumin (BSA) with the anionic surfactant sodium decylsulfonate ($C_{10}SO_3$), the cationic surfactant decyltriethylammonium bromide ($C_{10}NE$) and equimolarly mixed cationic–anionic surfactants $C_{10}NE$–$C_{10}SO_3$ were investigated by surface tension, viscosity, dynamic light scattering (DLS) and circular dichroism (CD). It was shown that the single ionic surfactant $C_{10}SO_3$ or $C_{10}NE$ has obvious interaction with BSA. The presence of $C_{10}SO_3$ or $C_{10}NE$ modified BSA structure. However, the equimolarly mixed cationic–anionic surfactants $C_{10}NE$–$C_{10}SO_3$ showed very weak interactions with BSA. The surface tension–log concentration (γ–$\log C$) plot for the aqueous solutions of $C_{10}NE$–$C_{10}SO_3$/BSA mixtures coincided with that of $C_{10}NE$–$C_{10}SO_3$ solutions. Viscometry showed that there is no significant change in the rheological properties for the $C_{10}NE$–$C_{10}SO_3$/BSA mixed solutions. DLS showed that BSA monomers and mixed aggregates of $C_{10}NE$–$C_{10}SO_3$ existed in the $C_{10}NE$–$C_{10}SO_3$/BSA mixed solutions. From CD spectra no obvious modification of BSA structure in the presence of $C_{10}NE$–$C_{10}SO_3$ mixtures was observed. The weak interactions between BSA and $C_{10}NE$–$C_{10}SO_3$ might be explained in terms of the very low critical micelle concentration (cmc) of $C_{10}NE$–$C_{10}SO_3$ mixtures that made the concentration of ionic surfactant monomers much lower than that needed for inducing the modification of BSA structure. In other words, the very strong synergism between oppositely charged cationic and anionic surfactants makes the formation of cationic–anionic surfactant mixed aggregates in the bulk solution a more favorable process than binding to proteins.
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1. Introduction

The interaction of proteins with surfactants has been a subject of extensive study for many years as it is of great importance in a wide variety of industrial, biological, pharmaceutical and cosmetic systems [1–11]. Studies on the interactions of surfactants with proteins can contribute to the understanding of the action of surfactants as denaturants and as solubilizing agents for membranes of proteins and lipids [1].

It is known in general that anionic surfactants interact strongly with proteins and form protein–surfactant complexes, which would induce the unfolding of proteins [1]. Cationic surfactants exhibit a lower tendency to interact with proteins [1]. In contrast to anionic and cationic surfactants, non-ionic surfactants bind very weakly to proteins [1]. It is attributed to the low critical micelle concentration (cmc) of some non-ionic surfactants and the absence of the electrostatic interaction between protein and non-ionic surfactant that make micelle formation in the bulk solution a more favorable process than binding to proteins [1,12]. In addition, the addition of non-ionic surfactants could reduce the interactions of anionic surfactants with proteins [1,12].

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Until now, the interactions of mixed cationic–anionic surfactants with proteins have not been reported. It might be that most of the equimolarly mixed cationic–anionic surfactants form precipitates or become turbid at very low concentration [13], which limits the research of interactions with proteins.

However, it is of great theoretical and practical importance to investigate their interactions. It is very often in practice that proteins and cationic–anionic surfactants are present in the same systems, especially in protein separation [14–17]. When cationic–anionic surfactants are used for protein separation, the interactions between cationic–anionic surfactants with proteins should be concerned, it should be required that the interactions are as weak as possible, so that the proteins can maintain their activity in the separating process.

In our previous paper [18], homogeneous solutions of equimolarly mixed anionic–cationic surfactants were reported. Aqueous mixtures of sodium decylsulfonate ($C_{10}SO_3$) and decyltriethylammonium bromide ($C_{10}NE$) can form homogeneous solution at any compositions even in high concentration, in which stable and narrowly distributed vesicles formed [18]. Such homogeneous systems allow investigating the interactions of proteins with cationic–anionic surfactants. In this work, the interactions of equimolarly mixed cationic–anionic surfactants $C_{10}NE$–$C_{10}SO_3$ with BSA were investigated by surface tension, viscosity, dynamic light scattering (DLS) and circular dichroism (CD), and were compared with those of single ionic surfactants with proteins.

2. Experimental

2.1. Materials

Bovine serum albumin (BSA) was purchased from Beijing Li-Ke Biochemistry Technology & Trade Co., Ltd. and had been purified by dialyzing and lyophilizing. Sodium decylsulfonate ($C_{10}SO_3$) was prepared from decane bromide and Na_2SO_3. The crude product was recrystallized from ethanol. Decyltriethylammonium bromide ($C_{10}NE$) was prepared from decane bromide and triethylamine. The crude product was recrystallized from mixed acetone–ether solvent. No surface tension minimas were found for $C_{10}SO_3$ and $C_{10}NE$, which implied that no surface-active impurities exist in them. Water was of Millipore quality.

2.2. Sample preparation

Stock solutions of BSA with different concentrations were prepared. The BSA concentrations were determined by UV-absorption measurements (Lambda 45 UV/Vis Spectrometer) at 280 nm using $A_{280\,nm}$ (1%) = 0.70.

2.3. Measurement of surface tension

The surface tension was measured by the drop volume method [19].

2.4. Viscosity measurement

The viscosity of protein–surfactant solutions was measured using an Ubbelohde capillary viscometer. The experimental temperature was maintained at 298 K.

2.5. Dynamic light scattering

DLS measurements were made using a spectrometer of standard design (ALV-5000/E/WINmultiple Tau Digital Correlator) and a Spectra-Physics 2017 200 mW Ar laser (514.5 nm wavelength). The scattering angle is $90°$, and the intensity autocorrelation functions were analyzed using the methods of Contin [20,21]. The experimental temperature was maintained at 298 K.

2.6. Circular dichroism

Far UV CD spectra were measured using a Jobin Yvon-Spex CD 6 at 298 K. Scans were obtained in a range between 200 and 260 nm by taking points at 0.5 nm, with an integration time of 1 s and a 2 nm bandwidth. Cells with path length of 0.1 mm were used.

3. Results and discussion

3.1. Surface tension

The studies of surface tension could provide evidence of interaction between proteins and surfactants [1]. When strong interactions between proteins and surfactants exist, the surface tension curve of protein–surfactant mixtures would deviate from that of the surfactants [1]. Figs. 1–3 show the surface tension curves of mixed systems of $C_{10}SO_3$, $C_{10}NE$ and $C_{10}NE$–$C_{10}SO_3$ with BSA. From Fig. 1, the curves show a decline in surface tension with the addition of BSA to $C_{10}SO_3$, suggesting the presence of BSA–$C_{10}SO_3$ complexes that have higher surface activity than $C_{10}SO_3$. Addition of BSA also declines the surface tension of $C_{10}NE$ solution (Fig. 2). However, Fig. 3 shows that the presence of BSA has a negligible effect on the surface tension of the $C_{10}NE$–$C_{10}SO_3$ solutions. The γ–log C plot of $C_{10}NE$–$C_{10}SO_3$/BSA mixtures coincides with that of $C_{10}NE$–$C_{10}SO_3$ solutions, which suggests that in the solution of $C_{10}NE$–$C_{10}SO_3$, no interaction of the surfactants with BSA can be detected. It has been shown that the surface tension curve of Triton X-100 and gelatin mixed solutions also coincides with that of the single Triton X-100 solutions [22]. Therefore, the interactions of cationic–anionic surfactants $C_{10}NE$–$C_{10}SO_3$ with BSA are similar to those
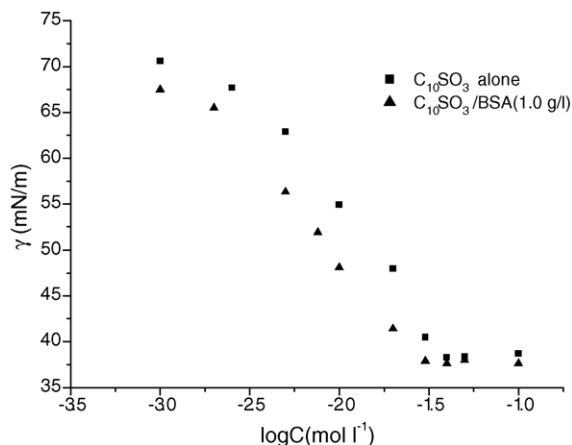
Fig. 1. Surface tension of $C_{10}SO_3$ in the absence and presence of BSA at 298 K; $\gamma_{BSA\ (1.0\ g/l)} = 68$ mN/m.
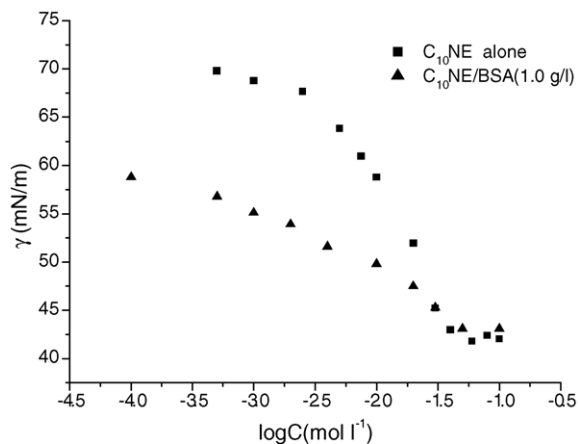

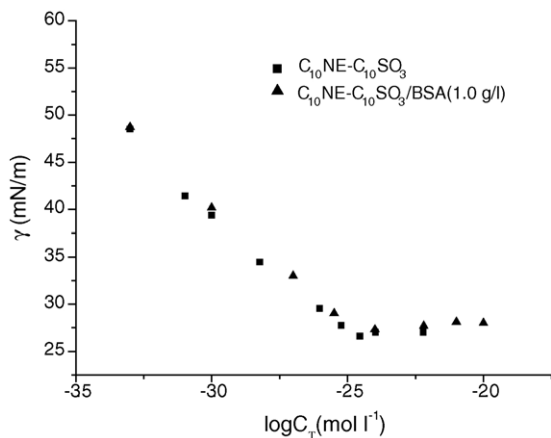Fig. 2. Surface tension of $C_{10}NE$ in the absence and presence of BSA at 298 K.

Fig. 3. Surface tension of $C_{10}NE$–$C_{10}SO_3$ in the absence and presence of BSA at 298 K. C_T represents the total concentration of $C_{10}NE$–$C_{10}SO_3$.
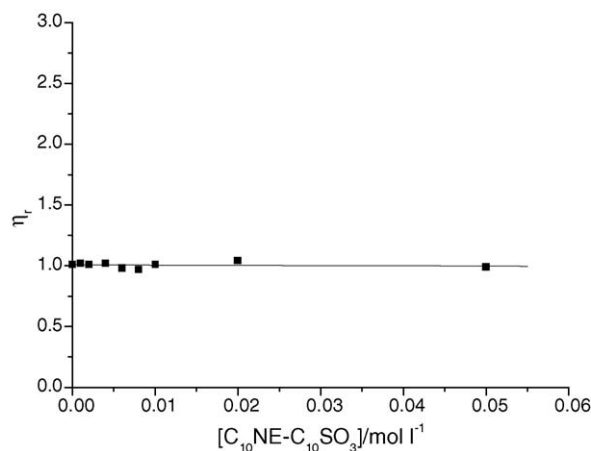

Fig. 4. The relative viscosity of $C_{10}NE$–$C_{10}SO_3$/BSA mixtures at 298 K; BSA concentration = 1.0 g/l.

of non-ionic surfactants with proteins in the sense of surface tension.

3.2. Viscosity

Viscometry is another effective method in probing conformational and rheological changes in interaction of protein with ionic surfactants [1]. Fig. 4 and Fig. 5 show the relative viscosity of $C_{10}NE$–$C_{10}SO_3$/BSA solutions, where the relative viscosity is defined as the ratio of the viscosity of the protein-doped solution to that of the protein-free solution. It could be seen from Fig. 4 and Fig. 5 that the addition of BSA to $C_{10}NE$–$C_{10}SO_3$ solutions has very little effect in viscosity, as the relative viscosity is very close to 1.00 in the mixed BSA and $C_{10}NE$–$C_{10}SO_3$ solutions, which suggests that equimolarly mixed $C_{10}NE$–$C_{10}SO_3$, up to 0.050 mol/l, do not form complexes with BSA that can result in significant changes in rheological properties. While for 0.050 mol/l $C_{10}SO_3$ or $C_{10}NE$ aqueous solution, the presence of BSA (2.0 g/l) results in an increase of 9% (for $C_{10}SO_3$) and 6%
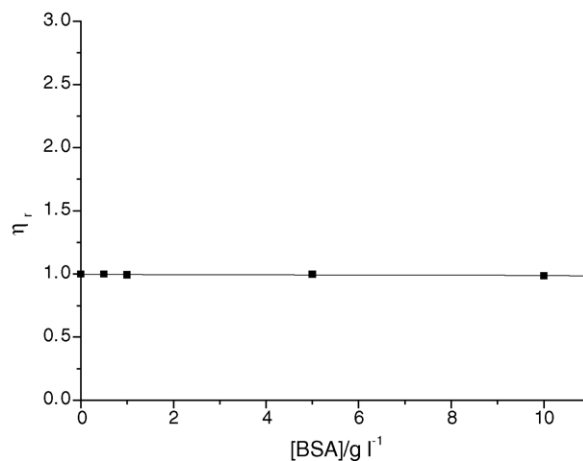

Fig. 5. The relative viscosity of $C_{10}NE$–$C_{10}SO_3$/BSA mixtures at 298 K; $C_{10}NE$–$C_{10}SO_3$ concentration = 0.010 mol/l.
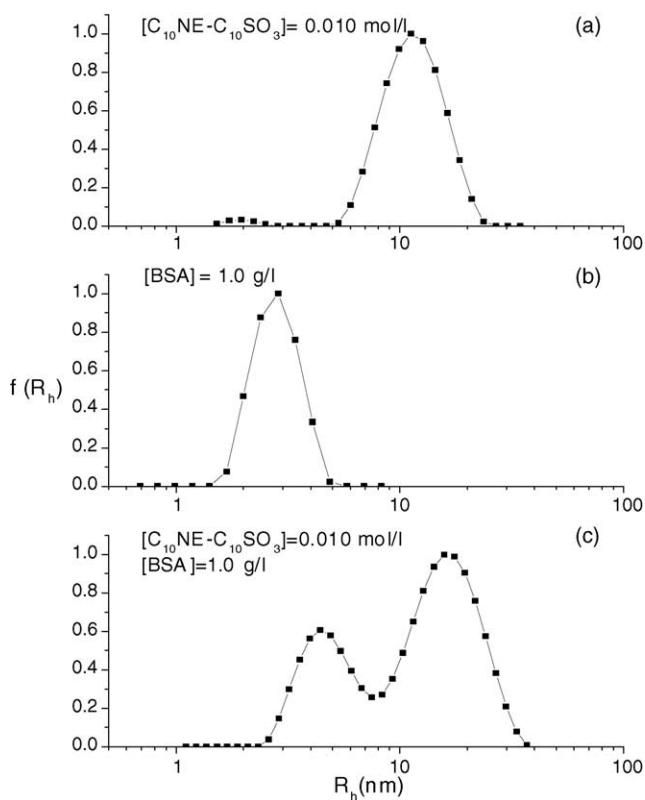
Fig. 6. The hydrodynamic radius (R_h) distribution of $C_{10}NE$–$C_{10}SO_3$, BSA and their mixtures at 298K. (a) [$C_{10}NE$–$C_{10}SO_3$] = 0.010 mol/l; (b) [BSA] = 1.0 g/l; (c) [$C_{10}NE$–$C_{10}SO_3$] = 0.010 mol/l, [BSA] = 1.0 g/l.

Fig. 7. The CD spectra of BSA in the presence of $C_{10}SO_3$ and $C_{10}NE$ at 298 K.

(for $C_{10}NE$) in the relative viscosity, implying the existence of BSA–$C_{10}SO_3$ complex or BSA–$C_{10}NE$ complex that results in the change in rheological properties of aqueous solution.

3.3. DLS

DLS can be used to estimate the hydrodynamic radii of the protein monomers (aggregates), surfactant aggregates and protein–surfactant complexes [2,23]. Fig. 6 shows the hydrodynamic radii distributions for $C_{10}NE$–$C_{10}SO_3$, BSA and their mixtures BSA/$C_{10}NE$–$C_{10}SO_3$. It can be seen from Fig. 6 that in $C_{10}NE$–$C_{10}SO_3$ and BSA mixed solutions, there exist BSA monomers and $C_{10}NE$–$C_{10}SO_3$ mixed aggregates, and no protein–surfactant complexes were detected.

3.4. CD

CD spectroscopy is a technique valuable for analyzing protein structure in solution [24]. Fig. 7 shows the effect of the single surfactant $C_{10}SO_3$ and $C_{10}NE$ on BSA structure. When the concentration of anionic surfactant $C_{10}SO_3$ is lower than cmc, it has little effect on BSA; when the concentration is higher than cmc, an obvious modification of BSA structure occurs. BSA shows some loss in the helix content as judged from the decrease in the negative ellipticity at 222 nm typical
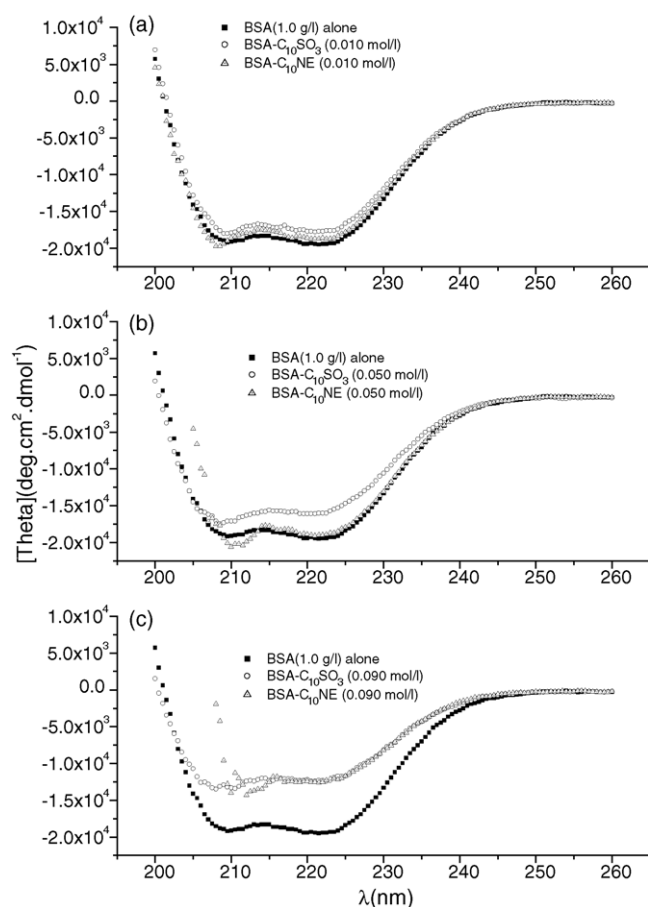
of α-helix [25]. This is in accord with the effect of SDS on the BSA structure [25]. At low $C_{10}SO_3$ concentrations, the surfactants molecules bind specifically to BSA and cause BSA to expand somewhat. While at high $C_{10}SO_3$ concentrations, $C_{10}SO_3$ molecules bind to BSA cooperatively and induce the unfolding of BSA. $C_{10}NE$ also causes the decrease of α-helix content of BSA at high concentration.

The CD spectra of BSA in the absence and presence of $C_{10}NE$–$C_{10}SO_3$ are shown in Fig. 8. It was shown that BSA maintained its native structure in the presence of different concentrations of $C_{10}NE$–$C_{10}SO_3$ (from below the cmc to above the cmc). It suggests that no protein–surfactant complex is formed.

Cationic and anionic surfactants manifest strong synergistic interactions when mixed in aqueous solutions [13]. Besides the hydrophobic interactions, there also exists the strong electrostatic attraction between the oppositely charged headgroups. Such a strong synergism induces the aggregations of surfactant molecules greatly, thus makes the cmc of the mixtures much lower than that of the individual components. As a result, at the cmc, the monomer concentrations in $C_{10}NE$–$C_{10}SO_3$ solutions are distinctly less than those in single component surfactant $C_{10}SO_3$ and $C_{10}NE$
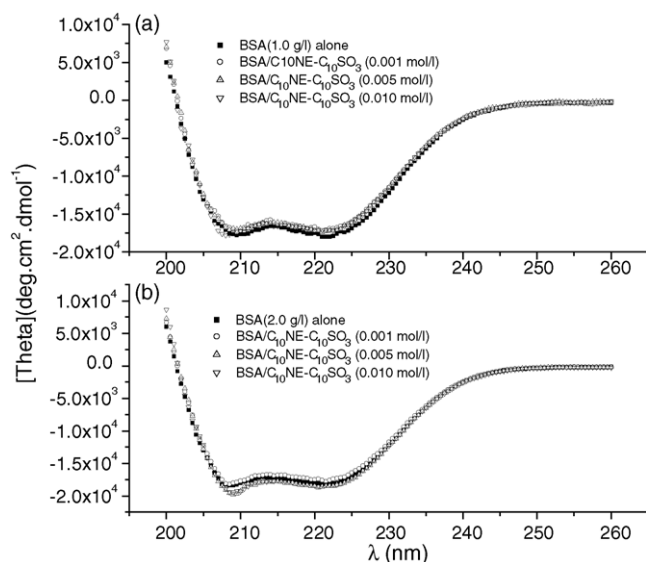
Fig. 8. The CD spectra of BSA in the presence of $C_{10}NE$–$C_{10}SO_3$ at 298 K.

solutions, which make the concentration of ionic surfactant monomers much lower than that needed for inducing the modification of BSA structure. In the mixed systems of BSA and $C_{10}NE$–$C_{10}SO_3$, almost all surfactants are in micellar state. Therefore, for the mixed systems of BSA and $C_{10}NE$–$C_{10}SO_3$, the formation of aggregates in the bulk would compete with the binding process, thus the binding of surfactant molecules to protein is limited by the formation of aggregates, and BSA maintains its native structure in $C_{10}NE$–$C_{10}SO_3$ solutions.

With the very high surface activity as well as rich and complex aqueous phase behavior [15], cationic–anionic surfactant mixtures are applied in many industrial areas, especially in the separation and purification of proteins [15]. It is the weak interaction of mixed cationic–anionic surfactants with proteins that they can be used for protein separation.

4. Conclusion

It was found that very weak interactions exist between BSA and equimolarly mixed $C_{10}NE$–$C_{10}SO_3$, which is distinctly different from the strong interactions of anionic surfactants with proteins. The salient features of cationic–anionic surfactants enable them to be used for protein separation with negligible effect on protein structure.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.colsurfb.2004.11. 011.

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