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Thermodynamics Study of Interaction Between β-Lactoglobulin with Sodium Dodecyl Sulfate, Dodecyl Trimethyl Ammonium Bromide and their Mixtures

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The interactions of bovine β-lactoglobulin (β-LA) with anionic surfac-tant sodium dodecyl sulfate (SDS), cationic surfactant dodecyl triethyl ammonium bromide (DTAB) and the mixtures of cationic-anionic surfactants (SDS-DTAB) have been carefully investigated by fluorescence, UV and circular dichroism (CD) techniques to elucidate the effect of these ligands on its structure. At pH 7.0 β-LA is negatively charged, therefore the absorbance of β-LA increased with the addition of SDS and the interaction of SDS with β-LA was the most distribution hydrophobic and a slight red shift of maximum peak position (from 275-278 nm) was observed, while DTAB had the different behaviour. The DTAB with β-LA had blue shift and the first electrostatic and the second hydrophobic interactions. Therefore UV showed increase and decrease in DTAB-β-LA absorption. SDS and DTAB and SDS-rich mixtures of SDS-DTAB form homogeneous solutions with β-LA CD observed that both SDS and DTAB could change the β-LA structure. The effects of the mixtures of SDS-DTAB on β-LA structure depended on the ratio of SDS-DTAB. DTAB and the SDS-rich of SDS-DTAB could significantly affect β-LA structure, while SDS exhibited weaker interaction with β-LA. The fluorescence study indicated a change in the environment of the tryptophan residue of β-lactoglobulin upon binding. Fluorescence quenching, from which binding parameters were evaluated, revealed that the quenching of the β-LA by SDS resulted from the formation of a SDS-β-LA complex. Fluorescence measurements showed that both SDS and DTAB could induce the quenching of fluorescence of β-LA, but DTAB enhanced the β-LA fluorescence when DTAB concentration was above 0.04 mM. The effect of the SDS-rich mixtures of SDS-DTAB on the fluorescence of β-LA became stronger with the increase of the molar fraction of SDS in SDS-DTAB mixtures.

Key Words: β-Lactoglobulin, Sodium dodecyl sulfate, Dodecyltriethyl ammonium bromide, Cationic-anionic surfactants, Circular dichroism, Fluorescence, UV spectroscopy.

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INTRODUCTION

Bovine milk proteins are among the most important food proteins consumed and utilized in terms of their nutritional value and functional characteristics. Bovine β-lactoglobulin (β-LA), were characterized and partially purified using electrophoretic, immunoblotting and chromatographic methods[1]. In this paper, the authors described protein folding studies in present surfactant and their mixtures. To elucidate protein-folding mechanisms, it is important to detect and characterize a kinetic folding intermediate(s) accumulated transiently during folding[2]. β-Lactoglobulin is a monomeric globular protein with molecular weight of 14200 Da and is one of the best-studied proteins in protein folding studies[2]. Nowadays, many researches on the binding of surfactants to proteins have been carried out, but the mixed surfactant systems limit the research of the interaction of mixture of cationic-anionic surfactants with proteins[3].

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[4]. In these studies the milk protein β-lactoglobulin (β-LA), which is the specify component of lactose synthesis in the lactating mammary gland[5,6] and has several partially folded intermediate states, which are being studied by many researchers interested in protein folding problems and it can induce apoptosis in tumor cells[7,8] which suggest that this protein can fulfill many important biological functions[9]. The reported studies were focused on the interaction of β-lactoglobulin with single surfactants[1], but rarely with the mixture of surfactants[10].

Among all the mixed surfactant systems, the mixtures of cationic-anionic surfactants are of particular interest. They exhibit much higher surface activity and much lower critical micelle concentration (CMC) than their individual components because of the strong electrostatic interactions between the opposite-charged head groups[11]. However, most of the equimolar mixtures of cationic-anionic surfactants form precipitate at very low concentration[11], which limits the research of the interaction of mixture of cationic-anionic surfactants with proteins. Our previous work found that the mixtures of sodium dodecyl sulfate (SDS) and dodecyl triethyl ammonium bromide (DTAB) could form homogeneous solutions at any compositions in high concentration, which allows to study the interaction between proteins and the mixture of cationic-anionic surfactants[12,13]. Surfactant-protein interaction is generally a combination of several interactions, among which the electrostatic and hydrophobic interactions play key roles[4]. It is known that the physico-chemical properties of the mixture of cationic-anionic surfactants relate to the molar ratio of cationic surfactant to anionic surfactant[11], so it is of significance to check the effect of the molar ratio of cationic surfactant to anionic surfactant on the interaction between protein and the mixture of cationic-anionic surfactants. In this work, we studied the interaction of β-lactalbumin with anionic surfactant SDS, cationic surfactant DTAB and the mixtures of anionic-cationic surfactants (SDS-DTAB). Circular dichroism and fluorescence

and UV were used to monitor the structural changes of protein when interacting with surfactants.

EXPERIMENTAL

Bovine β-lactoglobulin (β-LA) was obtained from Merck Chemical Co. β-Lactoglobulin was dissolved in the pH 7 buffer solution (2.817×10^{-5} M) and β-lactoglobulin stock solution was kept in refrigerator. Sodium dodecyl sulfate (SDS) and dodecyl triethyl ammonium bromide (DTAB) were also purchased from Merck Co. The NaH_2PO_4-Na_2HPO_4 buffer (pH 7, 0.02 mol/L) was used. The water was deionized, distilled. All other chemicals were of analytical reagents grade. The pH was recorded by metrohm 691 pH meter instrument. The two fields to which the protein circular dichroism (CD) spectroscopy is applied with well-developed methodology are folding thermodynamics[14,15] and secondary structure estimation[16]. Most thermodynamic studies rely on relative changes in CD spectra and, therefore, are relatively independent of calibration with structure[17]. Circular dichroism (CD) spectra were measured using a Jasco J-815 model spectropolarimeter with an integration time of 1 s and a 2 nm bandwidth. The two negative peaks of the elliptically at 208 and 222 nm in the CD spectrum of native β-lactalbumin in phosphate buffer are mainly due to tryptophan (four in bovine β-LA) absorbance[9]. The far-UV CD spectra are characteristic of the secondary structure of protein and the negative peaks of the ellipticity at 208 and 222 nm are typical of α-helical content of protein[18]. Measurements of steady-state fluorescence were made using Varyan Cary-Eclips spectrofluorometer model. Fluorescence spectroscopy was used to study the conformational changes of proteins when binding ligands, because the intrinsic fluorescence of tryptophan (Trp) residues is particularly sensitive to their micro-environments[19]. The excitation wavelengths were 280 and 292 nm and the emission spectrum was scanned from 300-460 nm. The integrated area of β-LA Trp fluorescence in the absence of surfactants was normalized at $F_0 = 1$. Each of the other integrations is the corresponding value of F at the different concentrations of surfactants. UV-vis absorption spectrum is a very simple method and applicable to explore the structural change and to know the complex formation[3]. UV measurements were performed at 25 K with Perkin-Elmer UV spectrophotometer using 1.0 cm path length quartz cuvette. Typical concentrations were of the order of 10^{-5} mol/L and the concentration of protein solution was measured by spectrophotometer an extinction coefficient of $E^{\%}$ (λ_{max} = 275 nm) = 1.7×10^{-6} M^{-1} cm^{-1}. Data were obtained at intervals of 2 min. The same experiments were performed three times to check reproducibility of the measurement.

RESULTS AND DISCUSSION

Circular dichroism spectra: Circular dichroism (CD) detection play an increasingly important role in the secondary structure of protein. Fig. 1 showed the CD spectra of β-lactoglobulin in the presence of SDS. When SDS was added to β-LA solutions, the ellipticity of β-LA at 208 and 222 nm increased (Fig. 1). When the

SDS concentration was increased to about 0.19 mM the peaks at 208 and 222 nm almost disappeared (the ellipticity of β-LA at 208 and 222 nm increased to near zero). It indicats that the Trp residues were in less constrained environment than in the native β-LA and the elimination positive area indicated inducted second structure β-LA and hydrophobic or electrostatic interaction. It meant that the α-helical content of β-LA was increased[20]; therefore, the secondary structure of β-LA was changed. Fig. 2 showed the CD spectra of β-LA in presence of DTAB. At about 0.1 mM of DTAB, the β-LA secondary structure was significantly changed (Fig. 2). Dodecyl triethyl ammonium bromide indicated that the first unfolding and then the α-helical content of β-LA increased. Fig. 3 showed the effects of the mixtures of SDS-DTAB on β-LA structure. Fig. 3 illustrated by the variation of the ellipticity at 208 nm, in the CD spectra of β-LA (the original CD spectra were shown in supplementary material). The increase of $[\theta]_{208}$ indicates the change of β-LA secondary structure. It was shown that the effect of SDS-DTAB mixtures on β-LA structure depended on the ratio of DTAB-SDS. The SDS-DTAB mixtures changed the essence of interaction of β-LA the ratio of only SDS did. It also indicated that the SDS-rich mixtures 4:1of SDS-DTAB could significantly affect β-LA structure, while the 3:1 mixtures of SDS-DTAB had weaker interaction with β-LA.

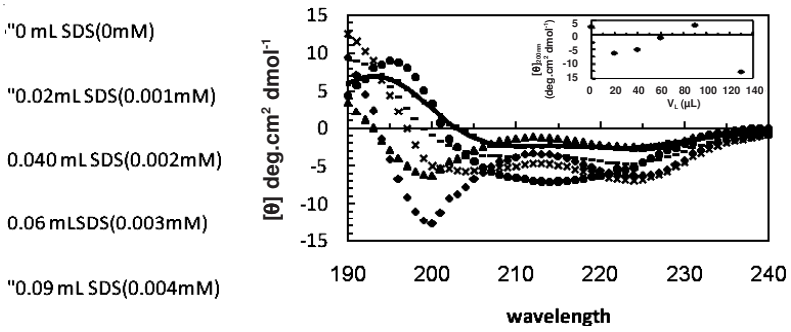


Fig. 1. Circular dichroism spectra of β-LA (0.04 %) in presence of different concentrations of SDS at 298 K in 20 mM phosphate buffer (pH 7)

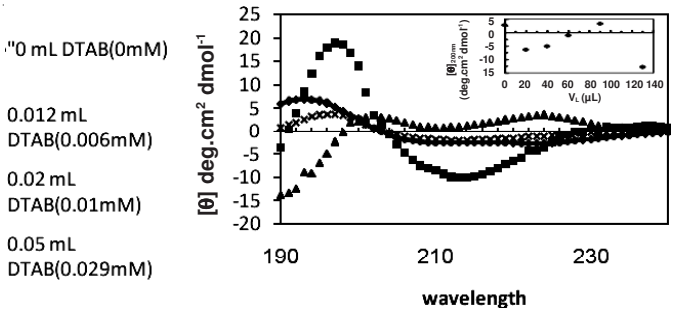


Fig. 2. Circular dichroism spectra of β-LA (0.04 %) in presence of different concentrations of DTAB at 298 K in 20 mM phosphate buffer (pH 7)

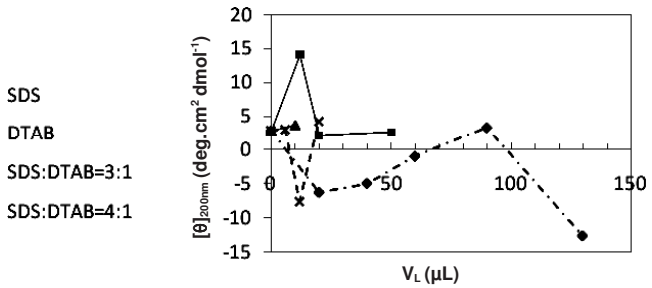


Fig. 3. Ellipticity of β-LA (0.04 %) at 208 nm in the presence of SDS-DTAB mixtures (molar ratio [SDS]/[DTAB] = 3/1 and 4/1) at 298 K in 20 mM phosphate buffer (pH 7). [L] is the total concentration of SDS and DTAB

Fluorescence spectra: Fluorescence spectroscopy is based on absorption of light by fluorophores within the biological tissue. The fluorophore then emits fluorescence radiation with specified spectrum. The in density and spectral shape of fluorescence depend on different factors, such as lesion depth and optical properties of the tissue. To evaluate fluorescence spectra it is essential to develop a mathematical model to describe variation of fluorescence intensity in dysplastic tissue[21]. Fluorescence quenching is a powerful method to study the molecular interactions involving proteins because it is sensitive, rapid and relatively easy to use[3]. Figs. 4 and 5 illustrate β-LA fluorescence intensity *versus* surfactants concentration. Present studies demonstrate that the spectra excited at 280 and 292 nm showed the same shape for SDS and SDS-DTAB mixtures, thus in Figs. 4 and 5 only the data of 280 nm were shown. At 280 nm, both tryptophan (Trp) and tyrosine (Tyr) contribute to the emission signal, whereas, using longer excitation wavelength (292 nm), the photo selection of Trp residues occurs[22]. As the spectra at the two wavelengths showed the same shape for SDS and SDS-DTAB mixtures, it could be thought that the contribution of Tyr was negligible. It might be owing to a very efficient energy transfer process from Tyr-Trp[22]. But for DTAB the spectra at the two wavelengths showed different shape, therefore, the contribution of Tyr was significant. Upon addition of surfactants SDS resulted in the decrease of β-LA fluorescence, corresponds to a polar environment of water solution. As the surfactant concentration increases, fluorescence intensity decreases rapidly, indicating that the β-LA is sensing a more hydrophobic environment. The fluorescence spectra of SDS usually show small stokes shifts. When the SDS was added into the β-lactoglobulin solution, a strong fluorescence appeared. The maximum emission wavelength of the SDS-β-LA complex is at 273 nm. It is observed that the fluorescence intensity increases, saturates and decreases by increasing DTAB concentration. The figure also shows that for larger concentration fluorescence intensity is smaller, that is because of higher attenuation of the interaction protein-surfactant. Dodecyl triethyl ammonium bromide established the blue shift of the emission maximum. This result is similar from those in lysozyme-surfactant systems or BSA-surfactant systems, in which the addition of surfactants quenches the fluores-

cence of protein[13,23]. Different results were also observed in BLG-SDS systems[24] and BLG-$C_{10}SO_3$ and BLG-C_{10}NE and $C_{10}SO_3$-C_{10}NE mixtures systems[10].

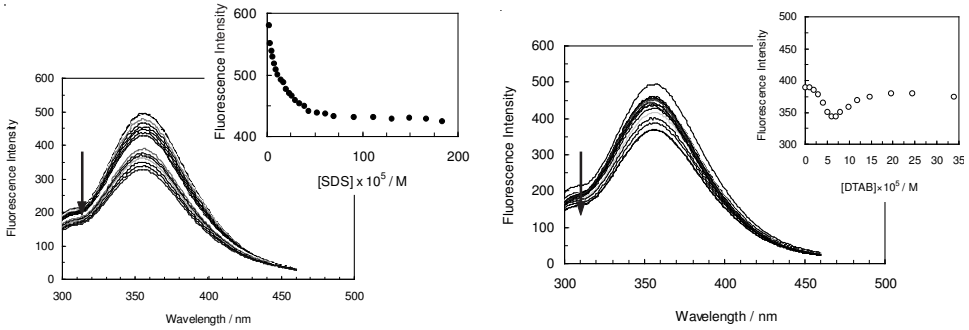


Fig. 4. Fluorescence spectra of β-LA in 20 mM phosphate buffer (pH 7) as the function of surfactant SDS (a) and DTAB (b) concentrations at 298 K. β-LA concentration: 0.04 %. The arrows indicate the sequence of spectral changes upon is inhibited with increasing of surfactant concentration, from 0-50 mM, corresponding a decrease in fluorescence intensity for SDS and a decrease then increase in fluorescence for DTAB. The excitation wavelength was 280 nm

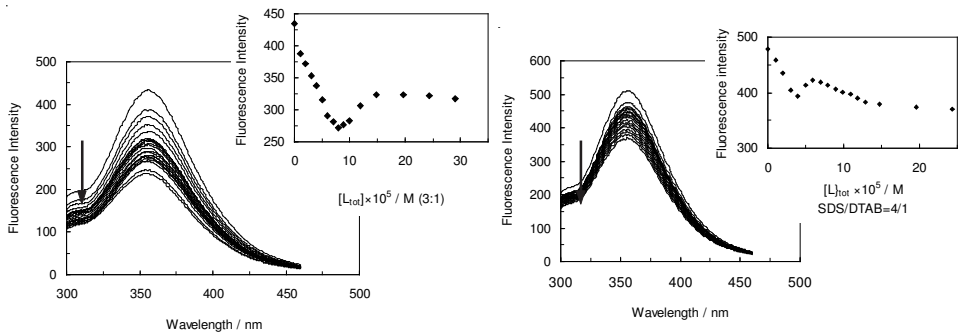
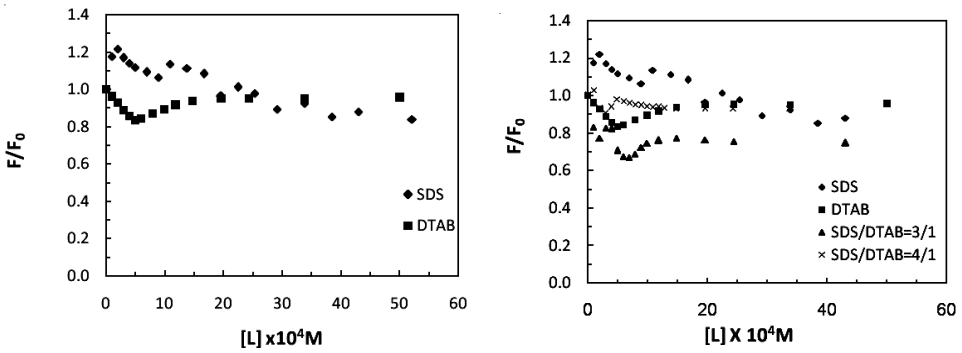


Fig. 5. Fluorescence spectra of β-LA in 20 mM phosphate buffer (pH 7.0) as the function of surfactant SDS-DTAB concentrations at 298 K. The molar ratios of SDS-DTAB are: (a) 3/1, (b) 4/1. β-LA concentration: 0.04 %. The arrows indicate the sequence of spectral changes upon increase of surfactant concentration, from 0 -0.50 mM (total concentration of SDS-DTAB), corresponding a decrease and then increase in fluorescence intensity. The excitation wavelength was 280 nm

UV spectroscopy: Figs. 6 and 7 showed UV spectra of β-LA in presence of surfactants. It was excited at 240 and 330 nm ranges. The broad peak with the maximum at 275 nm (connected with the presence of aromatic amino acids in protein molecule) is a characteristic feature of UV spectra for β-LA in phosphate buffer (pH = 7)[25] and it showed that β-LA absorption was due to tryptophan and

tyrosine. The addition of surfactants SDS resulted in the enhancement of β-LA absorbance and the red shift of the absorb maximum, thus its result was β-LA unfolding and hyperchromic effect. This result is different from those in DTAB and SDS-rich mixtures of SDS-DTAB, in which the addition of surfactants decrease the absorbance of protein. The absorption show clear dependence on surfactant.



(a) (b)

Fig. 6. Normalized areas of the spectra of β-LA as the function of surfactant concentrations at 298 K. β-LA concentration is 0.04 %. In (b) [L] is the total concentration for SDS-DTAB mixed systems

The changes of the UV-vis spectra with the addition of various surfactants are shown in Fig. 7. It is also indicated that the absorbance in UV spectra of β-LA to compare the effect of different surfactants and their mixtures on β-LA absorption. It was noticed from Fig. 7 that DTAB changed β-LA absorbance more strongly than SDS did. Absorbance in UV spectra of β-LA decreased to about 0.35 at the DTAB concentration of 0.02 mM, while increased about 0.01 for SDS at the same concentration. Fig. 7 also showed that the effect of SDS-DTAB depended on SDS molar fraction. The higher the molar fraction of SDS was the little the β-LA absorbance decreased. Thus, SDS-DTAB mixtures could be a way to modulate the effect DTAB on β-LA structure.

Most of β-lactoglobulin, consist of 123 amino acid residues. Only rat β-LA contains 17 additional C-terminal residues[9]. β-Lactoglobulin is homologous in sequence to the lysozyme family, but it exhibits cell lytic activity about 10^{-6} of hen egg white lysozyme[26]. X-ray crystallography has shown that the three dimensional structure of β-LA is similar to that of lysozyme[27,28]. Native consists of two domains: a large α-helical domain and small β-sheet domain, which are connected by a calcium binding loop (Fig. 8). The α-helical domain is composed of three major α-helices (residues 5-11, 23-24 and 86-98) and two short 3_{10} helices (residues 18-20 and 115-118). The small domain is composed of a series of loops, a small three-stranded antiparallel β-pleated sheet (residues 41-44, 47-50 and 55-56) and a short 3_{10} helix (three residues/turn and an intrachain hydrogen bond loop containing 10 atoms; residues 77-80). The two domains are divided by a deep cleft between them. At the

same time, the two domains are held together by the cysteine bridge between residues 73 and 91, forming the Ca^{2+} binding loop. A second important disulfide bridge 61-77 connects the two domains as well. Overall, the structure of β-LA is stabilized by four disulfide bridges (6-120, 61-77, 73- 91 and 28-111)[9].

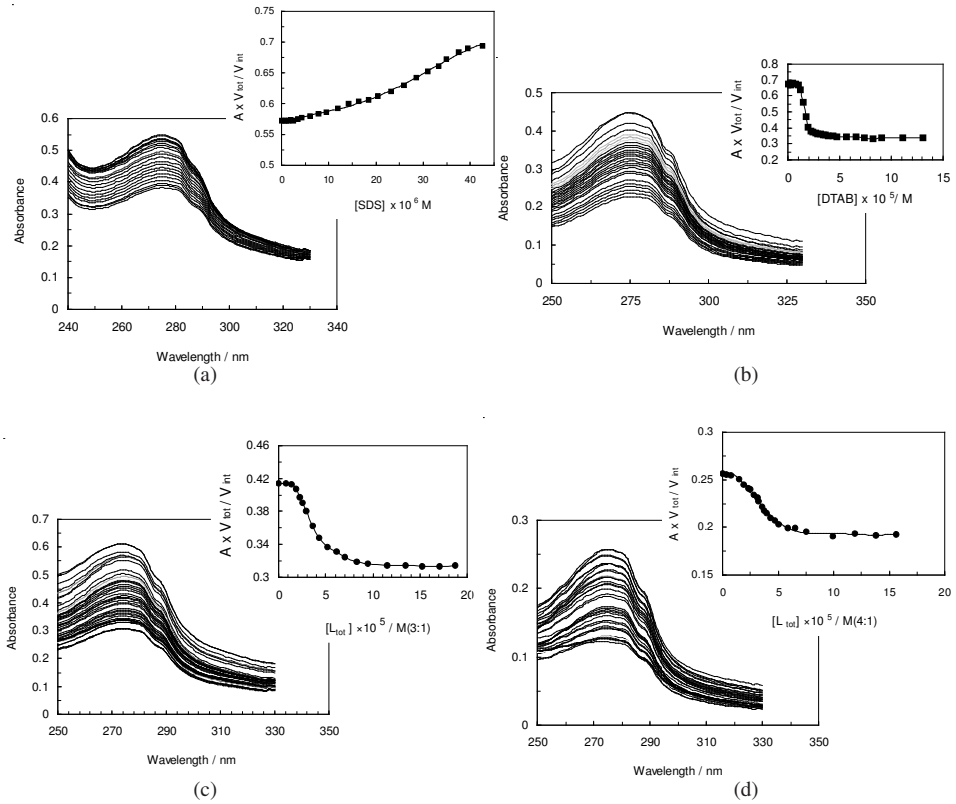


Fig. 7. UV curves of β-LA (0.04 %) in the presence of (a) SDS, (b) DTAB, molar ratio (c) [SDS]/[DTAB] = 3/1 mixtures and (d) [SDS]/[DTAB] = 4/1 at room temperature in 20 mM phosphate buffer (pH 7). [L_{tot}] is the total concentration of SDS and DTAB

When cationic surfactants are added into β-LA solution, the cationic surfactant monomers first bind electrostatically onto the negatively charged residues at the β-LA surface (site-specific binding) and this binding induces an expansion of the β-LA structure. This expansion, in turn, allows more interactions of the surfactant hydrophobic tails with the β-LA non-polar interior (nonspecific, cooperative binding) and leads to β-LA unfolding and loss of its secondary structure.

At pH 7.0, β-LA is negatively charged (the isoelectric point of β-LA is 4-5)[9], so it can form with oppositely charged surfactant DTAB owing to the formation of homogeneous neutral complexes of β-LA-cationic surfactant. With the further

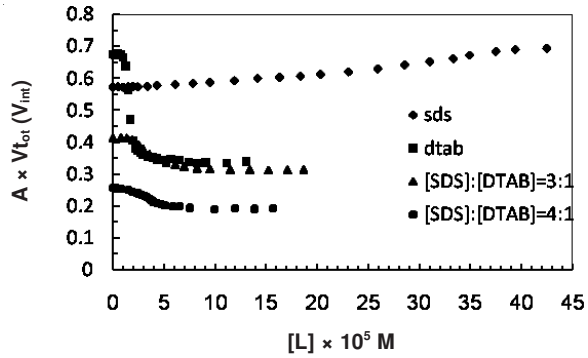


Fig. 8. Change of β-LA (0.04 %) in the presence of SDS, DTAB, and theirs mixtures (molar ratio [[SDS]/[DTAB] = 3/1 and 4/1) at room temperature in 20 mM phosphate buffer (pH = 7). [L] is the concentration of SDS and DTAB and the total their concentrations

increase of cationic surfactants, more cationic surfactants bind to the electrical neutral complexes, so the complexes of β-LA-cationic surfactant is positively charged. When anionic surfactant SDS binds to β-LA, the β-LA-SDS complex is always negatively charged, so homogeneous solutions were observed. The different binding model of DTAB and SDS to β-LA could be shown in their effect on the CD spectra, fluorescence and UV spectra of β-LA. Fig. 3 showed that the DTAB affect the β-LA structure significantly at about 0.059 mM. While SDS began to change the β-LA structure at about 20 mM, higher than DTAB did. The reason might be that β-LA contains more acidic amino acids than basic amino acids[10]. The fact that the opposite occurs is probably due to both ionic interaction of the negative charge of with the positive head groups of a single surfactant and hydrophobic effect due to its interaction with the spacer. The β-LA fluorescence is mostly related with the microenvironment of the Trp residues of β-LA[9,23]. β-Lactoglobulin contains four Trp residues[9]. In Fig. 9 the localization of the Trp residues in the native β-LA structure. Two Trps are in an apolar environment within the α-helical cavity of β-LA, whereas the one of α-helices tryptophanes protrudes and the β-sheet Trp beyond the surface of the molecule and is quite close to the S-S disulfide bridge[9]. As the disulfide bridge is an effective, Trp fluorescence quencher, the intrinsic fluorescence of β-LA is almost exclusively attributed to two Trps of within the α-helices[1,9,29]. The surfactant ions would tend to cluster around the oppositely charged side chains of proteins, *i.e.*, anionic surfactants cluster around Ca^{2+} and Zn^{2+} side chains and cationic surfactants around Glu 49 and Asp 116 side chains[9]. When DTAB concentration was above 0.04 mM, increased β-LA fluorescence intensity due to change in the environment of all emitting tryptophan residues[30]. Sodium dodecyl sulfate molecules bind to the residues of hydrophob, it has more effect on per four Trp. Therefore it strongly interacts with β-LA and it probably tends to unfold the protein structure[9], thus decrease the distance between Trps and the S-S disulfide bridges and strong

the quenching action of S-S disulfide bridge on Trp fluorescence, which could result in the decrease of the β-LA fluorescence intensity.

In the mixture of cationic-anionic surfactants, the critical micelle concentration is much lower than of the single components[11]. Consequently, in SDS-DTAB solutions, the surfactant aggregates form at lower concentration and the surfactant monomer concentrations are much lower than those in single components[10]. When the SDS-rich mixtures of [SDS]/[DTAB] = 3/1 are added to β-LA solution, the DTAB monomer concentration is enough to form complex with β-LA and no significantly change the β-LA structure and delayed protein unfolding. In the SDS-rich mixtures of [SDS]/[DTAB] = 4/1, DTAB monomer concentration is very low and not enough to form neutral complex with β-LA and the increase effect SDS concentration changed significantly the β-LA structure. However, the DTAB monomer concentration is high and DTAB could bind to β-LA and change β-LA structure, with the effect weaker than that of SDS-rich mixtures of [SDS]/[DTAB] = 4/1 but stronger than that of the SDS-rich mixtures of [SDS]/[DTAB] = 3/1. Therefore, by changing the molar ratio of SDS-DTAB, we could modulate the interaction of SDS-DTAB with β-LA.

Conclusion

We have studied the interaction of β-LA with SDS, DTAB and the SDS-rich mixtures of SDS-DTAB. In summary, at pH 7.0, β-LA could form complex with oppositely charged surfactant DTAB and SDS-rich SDS-DTAB mixtures and homogeneous solutions were observed in the mixtures of β-LA with surfactants and their mixtures. Present results showed that the effect of SDS-DTAB mixtures on β-LA structure depended on the ratio of SDS-DTAB, which could be a way to mediate the effect of surfactants on β-LA structure by changing the molar ratio of mixture of surfactants.

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