Interaction between Proteins and Cationic Gemini Surfactant

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Surface tension, fluorescence, and circular dichroism (CD) methods have been used to investigate the interaction between cationic gemini surfactant 1,2-ethane bis(dimethyldodecylammonium bromide) ($C_{12}C_2C_{12}$) and proteins including bovine serum albumin (BSA) and gelatin. Surface tension measurements show that the complexes of gelatin−$C_{12}C_2C_{12}$ form more easily than that of BSA−$C_{12}C_2C_{12}$. Addition of $C_{12}C_2C_{12}$ has a different effect not only on the polarity of the microenvironment in BSA and gelatin systems but also on their fluorescence spectra. It can be seen from far-UV CD spectra that the α-helical network of BSA is disrupted and its content decreases from 41.7% to 27.6% while the random coil content of gelatin increases from 53.0% to 55.9% with increasing $C_{12}C_2C_{12}$ concentration. The results from near-UV CD spectra show that the binding of $C_{12}C_2C_{12}$ induces changes of the microenvironment around the aromatic amino acid residues and disulfide bonds of BSA at high $C_{12}C_2C_{12}$ concentrations.

Introduction

Proteins are very important in living organisms and take part in almost all life processes. They can bind a wide variety of ligands such as bilirubin, fatty acids, hematin, metal ions, surfactants, and drugs.[1−9] Interactions of proteins with surfactants have been studied for many years because their mixtures have very important applications in biosciences, foods and cosmetics, drug delivery, detergents, and biotechnological processes. For example, blood serum is a mixture of human serum albumin with a number of compounds, including low-molecular surface-active molecules. The surface tension of such biological fluids is used as a diagnostic and therapeutic tool;[10] the complex between sodium dodecyl sulfate (SDS) and bovine serum albumin (BSA) is used in the determination of molecular weights of proteins by polyacrylamide gel electrophoresis.[11] Human hair and wool are two proteinacious substrates, which are frequently exposed to surfactants.

Many methods for studying the interaction between proteins and surfactants are continuously being developed, including surface tension,[12] potentiometry,[13] Brewster angle microscopy,[14] small angle neutron scattering,[15] etc.[16,17] Recently, using atomic force microscopy, Mackie et al.[18] investigated the displacement of *β*-lactoglobulin from the air/water interface by SDS. Ruso et al.[19] used electrical conductivity and dynamic light scattering methods to study the interaction of a fluorinated surfactant, sodium perfluorooctanoate, with lysozyme and to characterize the conformational transitions of lysozyme. Much investigation is focused on single-chain surfactants including cetyltrimethylammonium bromide (CTAB), SDS, and *tert*-octylphenoxypolyethoxyethanol (Triton X-100).[20−24] However, interactions of proteins with double-chain surfactants are less studied.

Gemini surfactant ($C_nC_mC_n$) consists of two hydrophobic chains, two polar headgroups, and a spacer linked at or near the head groups. It is superior to the conventional single-chain surfactant in many properties such as low Krafft temperature, low critical micelle concentration (cmc), and strong hydrophobic microdomain. Thus, it has received considerable attention.[25−30] Recently, using interfacial rheological methods, we have investigated the aggregation behaviors of gelatin with cationic double-chain surfactant 1,2-ethane bis(dimethyldodecylammonium bromide) ($C_{12}C_2C_{12}$) at the air/water interface.[31] In this work, the interaction of gelatin with $C_{12}C_2C_{12}$ is studied using surface tension, fluorescence, and circular dichroism (CD) methods. BSA is also studied for comparison.

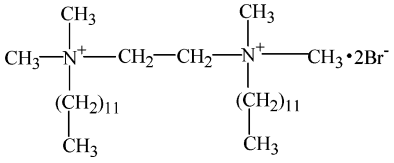

Figure 1. The structure of $C_{12}C_2C_{12}$.

Experimental Section

Materials. Bovine serum albumin (BSA) was obtained from Sigma. Gelatin (type B, 225 bloom) was purchased from China National Pharmaceutical Group with a sharp molecular weight distribution at 10^6 g·mol^{-1}. The isoelectric points of BSA[32] and gelatin[33] are 4.9 and ∼5, respectively. BSA and BSA−$C_{12}C_2C_{12}$ solutions were prepared by adding the desired amount of BSA to water at 25 °C. Gelatin and gelatin−$C_{12}C_2C_{12}$ solutions were prepared as described in ref 31.

Cationic gemini surfactant, $C_{12}C_2C_{12}$, was synthesized as described in refs 34 and 35, and its structure is displayed in Figure 1. The fluorescent probe, pyrene, was obtained from Sigma. Water used in the experiments was triply distilled by a quartz water purification system.

Surface Tension Measurements. Surface tensions were measured on Processor Tensiometer-K12 (Krüss Company, Germany) using the ring method. $C_{12}C_2C_{12}$ solution was kept for 15 min, while $C_{12}C_2C_{12}$−protein solution was kept for 1 h. When surface tensions did not change with time, the values were recorded. The average values of equilibrium surface tension were obtained by repeating three times.

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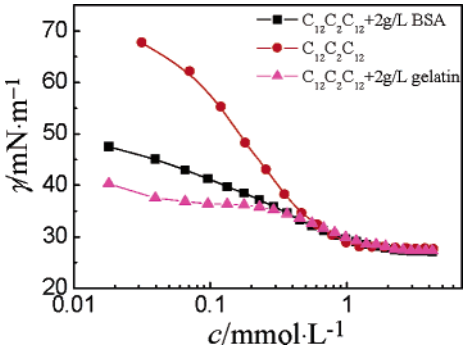

Figure 2. Surface tension isotherms of different solutions.

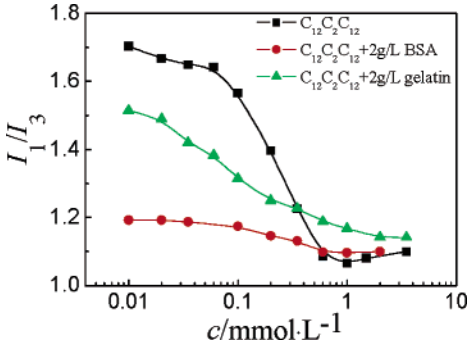

Figure 3. Dependence of I_1/I_3 on $C_{12}C_2C_{12}$ concentration in different solutions.

Fluorescence Measurements. Intensities and spectra of fluorescence were carried out on a Hitachi F-4500 fluorescence spectrophotometer using 1.0 cm quartz cells. The emission spectra of protein were monitored with a fixed excitation wavelength at 280 nm. However, pyrene spectra were recorded with fixed excitation wavelength at 335 nm. Intensities, I_1 and I_3, were taken from the emission intensities at 373 and 384 nm, respectively.

Synchronous fluorescence spectra were acquired by the same spectrofluorometer. They were recorded, keeping the difference between excitation wavelength and emission wavelength fixed ($\Delta\lambda = \lambda_{em} - \lambda_{ex}$). When the $\Delta\lambda$ is at 20 or 60 nm, the synchronous fluorescence gives the characteristic information of tyrosine (Tyr) residues or tryptophan (Trp) residues. All the excitation and emission slits were set at 10/2.5 nm.

Circular Dichroism Measurements (CD). Far-UV CD spectra were performed with a Jasco J-810 spectropolarimeter, using a bandwidth of 2.0 nm and a cell of 0.1 mm path length over the wavelength range between 190 and 250 nm. In the near-UV region, measurements were made a cell of 10 mm path length. Scans were made from 320 to 250 nm. The α-helical, β-sheet, and the random coil contents were analyzed using the curve fitting method of the far-UV CD spectrum with the Jasco secondary structure manager. All the experiments were performed at 25 °C.

Results and Discussion

Surface Activity of the System. Surface tension measurement is a simple and effective method of studying the interaction between macromolecules and surfactants. It is found from Figure 2 that the surface tension curve of the gelatin−$C_{12}C_2C_{12}$ system exhibits two break points, but that of the BSA−$C_{12}C_2C_{12}$ system does not. It is recognized that the first break point corresponds to the beginning of the formation of macromolecule−surfactant complexes in bulk, where concentration is known as the critical aggregation concentration (cac). The concentration at the second break point corresponds to the critical micelle concentration of the surfactant solution with the macromolecule. So it is deduced that the complexes of gelatin−$C_{12}C_2C_{12}$ form easily. On the other hand, although both gelatin−$C_{12}C_2C_{12}$ and BSA−$C_{12}C_2C_{12}$ systems show the lowering of surface tension at low surfactant concentration, the effect of gelatin−$C_{12}C_2C_{12}$ is more obvious. It is reported[10,36] that at low surfactant concentration a first interaction starts due to electrostatic force, and then protein interacts with surfactant via hydrophobic force as the surfactant concentration is increased. Eventually, their surface tensions agree with those of protein-free $C_{12}C_2C_{12}$ solutions, suggesting that gelatin or BSA molecules at the interface are gradually displaced by $C_{12}C_2C_{12}$ molecules due to competition in the adsorption layer.

Micropolarity. The intensity ratio of the first and the third major vibrational peaks in pyrene's fluorescence spectrum (I_1/I_3) is often used as a measure of the polarity of the microenvironment.[37−39] In agreement with the literature,[4] the I_1/I_3 value in water is 1.81. The I_1/I_3 value in the solution with 2 g·L^{-1} BSA is 1.27 while the I_1/I_3 value in the solution with 2 g·L^{-1} gelatin is 1.60. It can be found that pyrene tends to be located in a hydrophobic site formed by BSA or gelatin because the I_1/I_3 value in BSA or gelatin solution is much lower than in water. On the other hand, the I_1/I_3 value in gelatin solution is higher than that in BSA solution, suggesting that the polarity of the microenvironment in gelatin solution is higher than that in BSA solution. This phenomenon is ascribed to their structural differences. BSA has Trp, Tyr, and phenylalanine (Phe) residues while gelatin only has Tyr and Phe residues. It is well-known that the hydrophobicities of Trp, Tyr, and Phe are stronger than other residues, so the polarity of the microenvironment in BSA solution is weaker.

Figure 3 shows the change of I_1/I_3 value as a function of $C_{12}C_2C_{12}$ concentration in the absence and presence of protein. The I_1/I_3 curve shows a sharp transition at the cmc, which is around 0.9 mmol·L^{-1} for $C_{12}C_2C_{12}$ solution, decreasing from 1.70 to 1.10. Its cmc agrees favorably with the value obtained by the surface tension isotherm (Figure 2).

At low $C_{12}C_2C_{12}$ concentration, the I_1/I_3 value decreases considerably with the addition of BSA or gelatin. The I_1/I_3 value at high concentration is close to that of pure surfactant. For BSA−$C_{12}C_2C_{12}$ solution, pyrene prefers the hydrophobic zones of BSA at low surfactant concentration. A very small decrease in the ratio occurs, suggesting the formation of micelles, and pyrene migrates from BSA to the micelle because the polarities of the microenvironments in BSA and the micelle are similar. For the gelatin−$C_{12}C_2C_{12}$ system, noticeable changes in the I_1/I_3 value are observed in the presence of $C_{12}C_2C_{12}$, even at low surfactant concentration. The I_1/I_3 value decreases from 1.51 to 1.14 and approaches the value characteristic for the free micelles with an increased $C_{12}C_2C_{12}$ concentration, implying that the polarity of the microenvironments in gelatin is higher than that in the free micelle.

Fluorescence Spectra of the System. BSA has three intrinsic fluorophores, viz. Trp, Tyr, and Phe. Phe is not excited in most cases, and its quantum yield in proteins is rather low,[40] so the emission from this residue can be ignored. The intrinsic fluorescence of BSA is almost completely contributed by Trp alone because the fluorescence intensity of BSA for $\Delta\lambda = 60$ nm is higher than that for $\Delta\lambda = 20$ nm, as shown in Figure 4. It also can be seen that the fluorescence intensity of BSA decreases for $\Delta\lambda = 60$ nm and increases for $\Delta\lambda = 20$ nm when $C_{12}C_2C_{12}$ is added. However, the fluorescence intensity for $\Delta\lambda = 60$ nm decreases from 5273 to 4436 while it increases from 1901 to 2385 for $\Delta\lambda = 20$ nm at 0.1 mmol·L^{-1} $C_{12}C_2C_{12}$. This

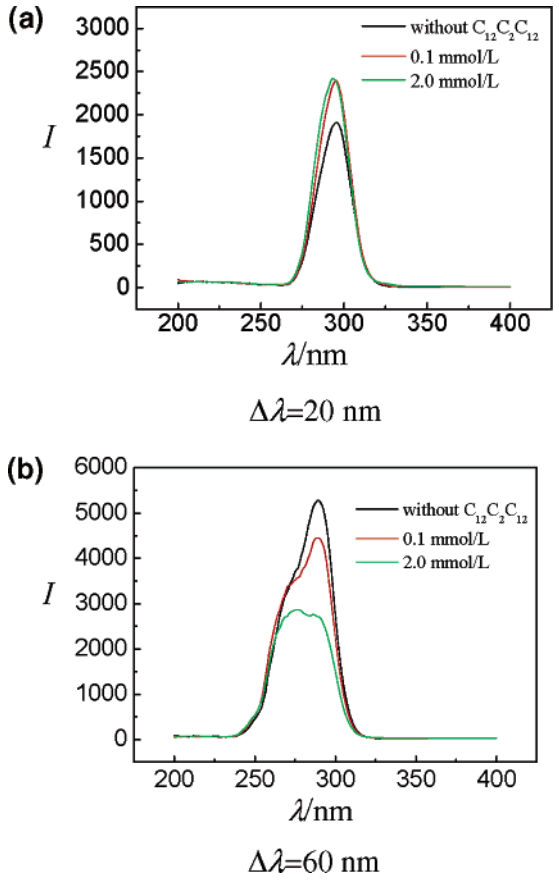

Figure 4. Synchronous spectra of BSA.

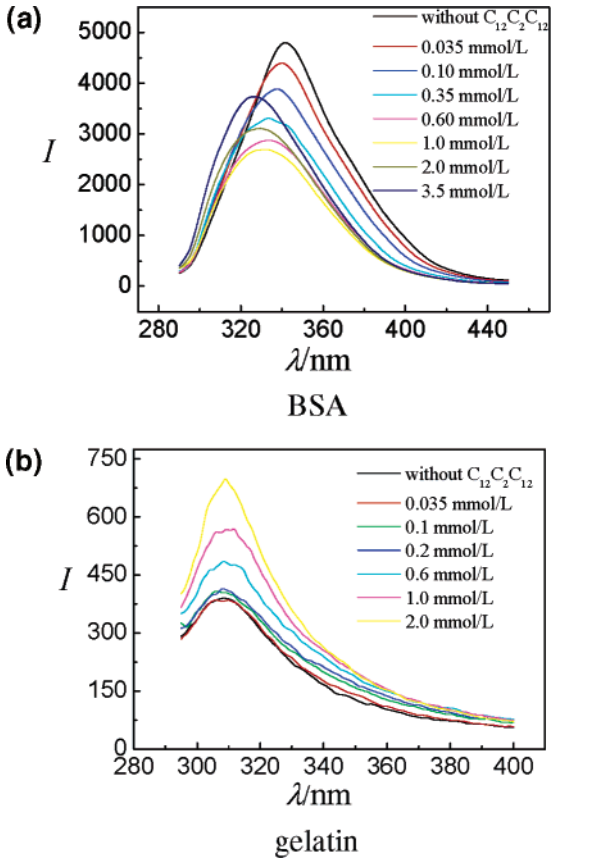

Figure 5. Fluorescence spectra of proteins at different $C_{12}C_2C_{12}$ concentrations.

indicates that $C_{12}C_2C_{12}$ mainly interacts with Trp residues compared to Tyr residues. So the emission peak of BSA is at 342 nm.

However, the fluorescence of gelatin results from Tyr because gelatin only contains two intrinsic fluorophores viz., Tyr and Phe, and has no Trp. So the emission peak of gelatin is at 308 nm.

First, it is seen in Figure 5a that addition of $C_{12}C_2C_{12}$ to BSA solution produces a decrease in the intensity of BSA fluorescence, and this is accompanied by a blue shift of the maximum emission peak, suggesting that Trp residues are exposed to a more hydrophobic environment. This phenomenon is ascribed to the formation of BSA−$C_{12}C_2C_{12}$ complex. Then, the fluorescence intensity increases gradually when $C_{12}C_2C_{12}$ concentration is above cmc (1 mmol·L^{-1}), which is due to partially denatured BSA. It is reported that a similar trend is also found in single-chain surfactant−BSA such as cationic CTAC−BSA and zwitterionic HPS−BSA.[41] However, when the concentration of $C_{12}C_2C_{12}$ is below 0.2 mmol·L^{-1}, the fluorescence spectra of gelatin change a little. A subsequent increase in $C_{12}C_2C_{12}$ concentration leads to a remarkable increase in the fluorescence intensity, but there is no change in peak position of gelatin (see Figure 5b).

Far-UV CD Spectra of the System. Far-UV CD measurements can give information about the secondary structure of proteins.[21,42] The far-UV CD spectra for BSA in the absence and presence of $C_{12}C_2C_{12}$ are shown in Figure 6a. The CD spectra of BSA exhibit two negative bands in the UV region at 208 and 218 nm, characteristic of an α-helical structure of protein. At low $C_{12}C_2C_{12}$ concentration (≤0.1 mmol·L^{-1}), there is little change in the helicity, suggesting that the secondary structure of BSA is possibly stabilized by $C_{12}C_2C_{12}$. However, at high $C_{12}C_2C_{12}$ concentration (3.5 mmol·L^{-1}), the percentage of helicity decreases from 41.7% to 27.6%, at the same time the β-sheet content increases from 22.8% to 37.2%, indicating that $C_{12}C_2C_{12}$ at high concentration disrupts the secondary structure and leads to the unfolding of BSA.

According to the literature,[43,44] the secondary structure of BSA may be stabilized by a cross-linking function at low concentration of $C_{12}C_2C_{12}$. Because $C_{12}C_2C_{12}$ has double tails, it maybe builds bridges between particular nonpolar residues and particular negatively charged residues located on different loops of BSA. At high concentration, the free micelles begin to form after the saturation binding of BSA with $C_{12}C_2C_{12}$, which leads BSA to an extended structure with exposed hydrophobic residues.

Unlike BSA, gelatin is a protein that is obtained by breaking the triple-helix structure of collagen.[45] Hence, the positive peak at 220 nm characteristic of the triple-helix disappears completely and the CD spectra of gelatin only exhibit the negative peak at 200 nm after complete denaturation of collagen. The negative peak at 200 nm corresponds to the random conformation. Addition of $C_{12}C_2C_{12}$ to gelatin solution does not alter the CD spectra significantly when its concentration is below 0.6 mmol·L^{-1}, but the content of the random coil increases from 53.0% to 55.9% at 2.0 mmol·L^{-1} $C_{12}C_2C_{12}$, leading to a more random structure. In response to this, $C_{12}C_2C_{12}$ addition also has a considerable effect on the percentage of β-sheet.

Near-UV CD Spectra of the System. The near-UV CD spectra of protein arise principally from aromatic amino acid residues and disulfide bonds.[43,46] Therefore, it is a useful probe for the tertiary structure of protein. As shown in Figure 7, the near-UV CD spectra of BSA around 250−320 nm have no remarkable change at low concentration (0.1 mmol·L^{-1}) of

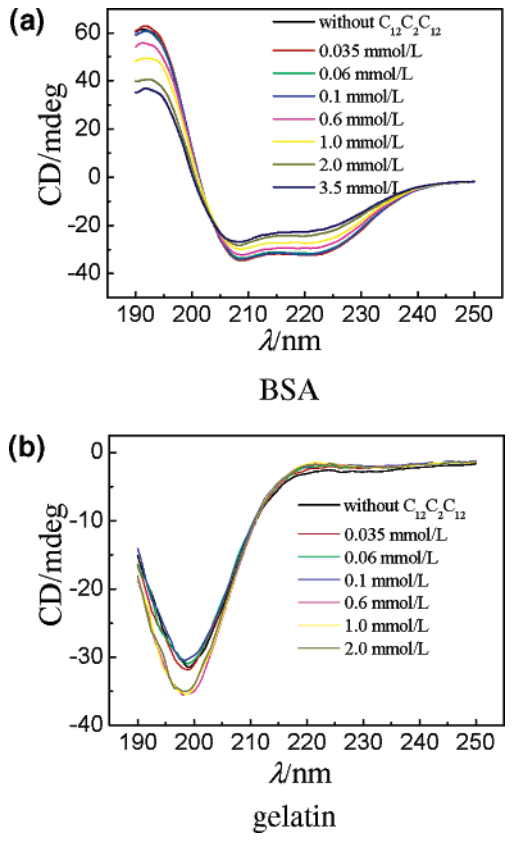

Figure 6. Far-UV spectra of proteins at different concentrations of $C_{12}C_2C_{12}$.

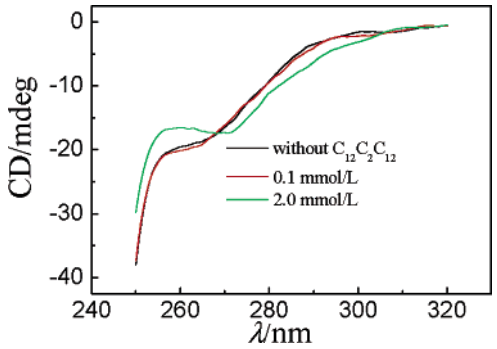

Figure 7. Near-UV spectra of BSA at different concentrations of $C_{12}C_2C_{12}$.

$C_{12}C_2C_{12}$. When the concentration of $C_{12}C_2C_{12}$ is 2.0 mmol·L^{-1}, an increase from 250 to 270 nm and a decrease from 270 to 300 nm in the ellipticity imply that the binding of BSA to $C_{12}C_2C_{12}$ induces the changes of the microenvironment around the aromatic amino acid residues and disulfide bonds. Drawing from the literature,[21] we come to the conclusion that BSA is less compact compared with the native state under this condition, which is consistent with the results obtained by the far-UV CD spectra.

Conclusion

$C_{12}C_2C_{12}$ with two hydrophobic chains and double charges can interact with gelatin and BSA through electrostatic and hydrophobic forces, leading to the changes in the fluorescence spectra of proteins and the polarities of the microenvironments. It is also found that the gelatin−$C_{12}C_2C_{12}$ system has two obvious breaks but BSA−$C_{12}C_2C_{12}$ does not in surface tension isotherms. Moreover, at a high concentration of $C_{12}C_2C_{12}$, gelatin or BSA at the interface is replaced by surfactant. At the same time, the conformational changes in proteins are monitored by CD spectra.

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