Analysis of Protein-Mixed Surfactant System Interactions; The BSA-SDS and Polyoxyethylenealkylether System

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Abstract: The denaturation of bovine serum albumin (BSA) by mixed surfactant systems was studied by examining surfactant adsorption on BSA. Polyoxyethylenealkylether (alcohol ethoxylate: AE) inhibited SDS-binding to BSA with consequent denaturation of BSA. The higher the concentration of AE or the larger the adduct number of oxyethylene (EO), the greater was the inhibitory effect of AE on SDS-induced denaturation of BSA. The adsorption of AE on an electrostatic surface significantly increased when SDS was present. The enthalpy of adsorption of the SDS-AE mixed system on BSA as determined with a microcalorimeter clearly differed from that of pure SDS or AE. The AE effect on SDS-induced BSA denaturation is thus due to the adsorption of AE on the alkyl chain of SDS electrostatically bound to BSA. The adsorption of AE apparently stabilizes the alkyl chain of SDS electrostatically bound to BSA, with a consequent decrease in the binding of SDS to BSA.

Key words: denaturation, adsorption, bovine serum albumin, alcohol ethoxylate, microcalorimeter

1 Introduction

Since the correlation between protein denaturation and skin roughness was shown in the early 1970s, protein denaturation has been used as a model for estimating skin roughness by surfactants and detergents. Regarding protein denaturation induced by surfactants, the interaction between surfactants and proteins has been studied by analysis of the characterization of surfactant binding and protein unfolding since the 1940s. Especially, concerning the interaction between SDS and bovine serum albumin (BSA), many studies have been done, for example, the binding isotherm, thermodynamics and the change in structure. It is also well-known that SDS-induced denaturation of BSA can be counteracted by dodecyltrimethylammonium chloride or N,N-dimethyldecylaminoxide. They hinder anionic surfactants from binding to BSA by forming a complex of two or more surfactants and neutralizing the electrical charge of the anionic surfactants such as SDS. The nonionic surfactants of the alkylene oxide adduct type, typically represented by alcohol ethoxylate (AE) and nonyl phenol ethoxylate, can also reduce SDS-induced BSA denaturation. It is known that the typical adsorption of surfactants on hydrophilic surfaces like that of alumina shows cooperativity, which indicates the formation of micelle-like aggregates of adsorbed surfactants. These aggregates are frequently called admicelles whose formation is driven by hydrophobic interactions between the tail groups. Mixed admicelles form at a surfactant concentration lower than that of admicelles of either pure component. In this paper, we investigated the interactions between BSA and the mixed system of SDS and AE by examining the BSA denaturation and the binding behavior of surfactants. The amount of SDS bound to BSA was decreased by the addition of AE. It is considered that AE forms admicelles with SDS on BSA, thereby reducing the amount of SDS electrostatically bound to BSA.

2 Experimental

2.1 Materials

BSA was purchased from the Sigma Chem. Co. N-(Iodoacetyl)-N’-(1-sulfo-5-naphthyl) ethylenediamine (1,5-Iaedans) was purchased from the Aldrich Chem. Co. SDS was obtained from the Wako Chem. Co. and further recrystallized 3 times.
from ethanol. Each AE (polyoxyethylene-dodecylether: EO adduct number=6 or 8) was obtained from the Nikko Chemicals Co. Various AEs with narrow EO distributions (average EO adduct number=5, 7, 9, 12, 15) were synthesized using a MgO catalyst modified by metal cations\(^{14}\).

BSA immobilized-agarose (BSA-agarose) was purchased from the Elastin Products Company, Inc. Q-Sepharose was purchased from the Amersham Pharmacia Biotech Co.

### 2.2 Measurement of circular dichroism of BSA solution

The surfactant solution was added to 3 \(\mu\)M BSA/50 mM phosphate-buffered solution (pH 7.0) so that the final concentration of the surfactant was 0.001-100 mM. To ensure complete interaction, the mixed solutions were allowed to stand overnight at 25°C. The spectrum near 220 nm (reflecting the \(\alpha\)-helix of BSA) was then measured for each aqueous solution using a circular dichroism dispersion meter (JASCO J-720). The change ratio of the \(\alpha\)-helix of BSA in the solution is detectable as the change ratio of the 220 nm peak value and was used as the BSA denaturation ratio\(^{14}\).

### 2.3 Preparation of BSA labeled by 1,5-IAEDANS\(^{15,16}\)

1,5-IAEDANS is a thiol-specific reagent whose reaction involves the release of hydrogen iodide. The solution of 0.1% BSA in 100 mM Tris-HCl buffer, pH 7.5, was incubated with 23 mM 1,5-IAEDANS in the dark at room temperature for 3 h. The mixture was then extensively dialyzed against 10 mM sodium phosphate buffer, pH 7.0, at 4°C and finally against 50 mM sodium phosphate, pH 7.0, at 4°C to remove any unreacted fluorophore.

### 2.4 Measurement of fluorescence of BSA-AEDANS

The surfactant solution was added to 200 ppm of BSA-AEDANS (BSA labeled with 1,5-IAEDANS)/50 mM phosphate-buffered solution (pH 7.0); then the mixture was allowed to stand for 24 hours at room temperature. The fluorescent intensity (Ex. 340 nm, Em. 480 nm) was measured for each aqueous solution using a fluorescence spectrophotometer (Hitachi).

### 2.5 Measurement of amount of surfactants adsorbed on BSA-agarose or Q-Sepharose

The amount of surfactants bound to BSA-agarose or Q-Sepharose was measured as follows. Several concentrations of SDS or AE and 1.2 mg/mL of BSA-agarose or 10 mg/mL Q-Sepharose were incubated with gentle shaking at room temperature for 30 minutes. The mixture was then centrifuged at 2,000 rpm for 10 minutes and the supernatant was collected. The supernatant was applied on the Nucleosil 100-5SB (for SDS, 4.6 × 250 mm, GL Science, Inc.) or Inersil ODS-2 (for AE, 4.6 × 150 mm, GL Science, Inc.). Using high performance liquid chromatography (Tosoh Co.), each surfactant was separated and quantified by refractometry.

### 2.6 Calorimetric analysis of surfactant adsorption on BSA

Isothermal titration microcalorimetry (MicroCal Co.) was employed. The enthalpy of the adsorption of surfactants on BSA was measured in 50 mM phosphate buffer (pH 7.0) at 25°C.

### 3 Results and Discussion

#### 3.1 The effect of C\(_{12}\)E\(_8\) on the SDS-induced denaturation of BSA

In order to investigate the SDS-induced denaturation of BSA in detail, we used a BSA-AEDANS which can sensitively estimate the unfolding of BSA. The AEDANS residue shows a sensitive response of both fluorescent intensity and emission wavelength when its hydrophobicity\(^{15}\), that is, the structure of BSA, is changed. The fluorescence emission wavelength decreased upon the addition of SDS to BSA-AEDANS. This phenomenon suggests that the hydrophobic environment of the region labeled with 1,5-IAEDANS became gently hydrophilic with the addition of SDS. Compared with the CD measurement, the denaturation of BSA by a lower concentration of SDS could be measured using BSA-AEDANS (Fig.1). Each denaturation ratio of BSA was calculated from the value of BSA which was sufficiently denatured by 30 mM SDS. This denaturation curve of BSA-AEDANS shows two phases: In the first phase, it is believed that the change in only the tertiary structure of BSA occurs, because the \(\alpha\)-helix ratio does not decrease. In the second phase, the significant reduction of the fluorescent intensity corresponds to a change in the secondary structure of BSA by the cooperative binding of SDS.

Figure 2 shows the BSA denaturation as a
Fig. 1 Denaturation of BSA as a Function of Concentration of SDS. pH 7.0, 50 mM phosphate buffer. ○: measured by CD ●: measured by fluorescent-labeled BSA

Fig. 2 Denaturation of BSA as a Function of Ratio of C₁₂E₈ to SDS. 5.6 mM SDS, pH 7.0, 50 mM phosphate buffer. ○: measured by CD ●: measured by fluorescent-labeled BSA

Fig. 3 Adsorption of SDS on Q-Sepharose from a Mixture of SDS and C₁₂E₈. The molar ratio of SDS to C₁₂E₈ was 1:2. ○: pure SDS ●: C₁₂E₈/SDS

Fig. 4 Adsorption on Q-Sepharose of C₁₂E₈ from a Mixture of SDS and C₁₂E₈. The molar ratio of SDS to C₁₂E₈ was 1:2. ○: pure C₁₂E₈ ●: C₁₂E₈/SDS

function of the concentrations of SDS which was given by the method of BSA-AEDANS applied to the SDS-C₁₂E₈ mixed system. The greater the concentration of AE, the greater was the reduction of BSA denaturation. The BSA denaturation was not, however, completely suppressed by the addition of C₁₂E₈. These results suggest that SDS can bind to BSA in the presence of AE though the binding rate is decreased more compared with the case in the absence of AE.

3.2 The adsorbing behavior of SDS and AE on the hydrophilic surface

In order to investigate the effect of AE on the SDS-induced denaturation of BSA, we first examined the adsorption of the SDS-AE mixture on a hydrophilic surface which is not denatured by SDS. As the hydrophilic surface, we used Q-Sepharose, an anion exchange resin with trimethylammonium residues. Figure 3 shows the amount of SDS which is bound to Q-Sepharose as a
function of the equilibrium SDS concentration. The amount of bound SDS increased as the SDS concentration became higher, and 1 g of Q-Sepharose was saturated with 0.2 mmol SDS. When C_{12}E_8 and SDS were in a ratio of 2:1, the binding of SDS was saturated at the equilibrium concentrations of 2 mM, and the amount of saturated SDS-binding was 0.13 mmol/g of Q-Sepharose. The amount of C_{12}E_8 adsorbed under the same condition is shown in Fig. 4. Though pure C_{12}E_8 was hardly adsorbed on Q-Sepharose, C_{12}E_8 could be adsorbed in the presence of SDS. This result indicates that SDS and C_{12}E_8 formed admicelles. In the absence of C_{12}E_8, SDS first electrostatically binds to Q-Sepharose, then SDS cooperatively adsorbs on Q-Sepharose by a tail-to-tail interaction, forming local bilayers or local monolayers (admicelles). Their formation is driven by hydrophobic interactions between the tail groups. In the presence of C_{12}E_8, C_{12}E_8 adsorbs on the alkyl chain of SDS, replacing SDS which is cooperatively adsorbed, so that the total SDS adsorption is reduced.

3.3 Adsorption behavior of SDS on BSA-agarose

We also examined the adsorption of an SDS-AE mixture on BSA. We used BSA-agarose to estimate the surfactant adsorption conveniently. The binding isotherm of SDS in the absence or presence of C_{12}E_8 onto BSA-agarose as a function of the equilibrium SDS concentration is shown in Fig. 5. Because SDS hardly bind to agarose beads (data not shown), it is considered that SDS binds to the BSA. In the absence of C_{12}E_8, the binding isotherm of SDS to BSA-agarose showed a pattern similar to that of SDS and BSA (Fig. 5; ○). Under the saturation binding condition, about 100 molecules of SDS bound to 1 molecule of BSA in the case of using BSA-agarose, whereas the typical saturation binding of SDS to BSA is more than 160 molecules of SDS/molecule of BSA. We considered two reasons for the difference between the SDS binding amount on native BSA and that on BSA-agarose. One is the suppression of the unfolding of BSA, because BSA molecules are immobilized on the agarose beads. The other is the decrease in SDS binding near the site of BSA which was immobilized on the agarose bead due to steric hindrance. In the presence of C_{12}E_8, the higher the C_{12}E_8 concentration, the lower the amount of SDS bound to BSA-agarose. This reduction of the amount of SDS bound to BSA-agarose by the addition of C_{12}E_8 was more significant than in the case of Q-Sepharose. The effect of C_{12}E_8 indicates that C_{12}E_8 is adsorbed on the alkyl chain of SDS which is electrostatically bound to the BSA, like admicelles. It is considered that the adsorbed C_{12}E_8 reduced the amount of electrostatic SDS-binding, so that the cooperative binding of SDS as well as the denaturation of BSA was dramatically suppressed by the addition of C_{12}E_8.

3.4 The effect of the EO chain length on the SDS-binding to BSA and the denaturation

We investigated the amount of surfactants adsorbed on the BSA-agarose under several conditions of concentration and average EO adduct number of AE. The amount of SDS-binding to BSA-agarose tended to be smaller as the average EO adduct number increased (Fig. 6). BSA denaturation by SDS closely paralleled the adsorption behavior (Fig. 7). As for the adsorption of AE on BSA, when the average EO adduct number was more than 9, the amount of every AE adsorbed on BSA was almost the same.

3.5 The enthalpy change in surfactants binding to BSA

It is well-known that about ten high energy
Fig. 6 Adsorption of SDS and \( \text{C}_{12}\text{E}_n \) from Their Mixture on BSA-agarose. \( \text{C}_{12}\text{E}_n \) : narrow EO distribution (average EO adduct number = 5, 7, 9, 12, 15). pH 7.0, 20 mM phosphate buffer.

- : SDS (1 mM SDS/0.2 mM \( \text{C}_{12}\text{E}_n \))
- : SDS (1 mM SDS/1 mM \( \text{C}_{12}\text{E}_n \))
- : \( \text{C}_{12}\text{E}_n \) (1 mM SDS/0.2 mM \( \text{C}_{12}\text{E}_n \))
- : \( \text{C}_{12}\text{E}_n \) (1 mM SDS/1 mM \( \text{C}_{12}\text{E}_n \))

Fig. 7 Denaturation of BSA by SDS and \( \text{C}_{12}\text{E}_n \) Mixed System. 1 mM SDS and indicated concentrations of \( \text{C}_{12}\text{E}_n \) with narrow EO distribution (average EO adduct number = 5, 7, 9, 12, 15). pH 7.0, 20 mM phosphate buffer.

- : \( \text{C}_{12}\text{E}_n = 0.2 \text{ mM} \)
- : \( \text{C}_{12}\text{E}_n = 1 \text{ mM} \)

binding sites of SDS exist on BSA. The cooperative binding of SDS on BSA is induced when more than about 10 SDS molecules bind to BSA; \( \Delta H \) then sharply increases and the denaturation of BSA, which can be detected by a fluorescent-labeled method, gradually starts. To investigate the interaction between a few molecules of SDS and the BSA molecule in detail, the enthalpy change for the high energy binding and the cooperative binding of some molecules of SDS to BSA was calorimetrically studied. Figure 8 shows the calorimetric data for SDS binding to BSA which were measured by isothermal titration microcalorimetry. When the molar ratio of SDS per molecule of BSA was less than 20, the enthalpy change titrimetrically decreased. Above (SDS molecules/molecule of BSA) = 20, the enthalpy curve decreases; that is, the interaction between SDS and BSA becomes exothermic again, indicating that the enthalpy change due to both the change in the structure of BSA and the adsorption of SDS on the sites of BSA, which were newly exposed, was detected. The difference in enthalpy between native BSA and fluorescent-labeled BSA is also shown in Fig. 8. Interestingly, the enthalpy curve of the native BSA has a slow rising section at about 5 moles of SDS per mole of BSA. This result indicated that there are two patterns for the high energy binding sites of SDS on native BSA, while
Fig. 9 Adsorbing Enthalpy for Pure or a Mixture of SDS and C12E8 on BSA. In SDS and C12E8 mixed system, the adsorbing enthalpy is shown as a function of molar ratio of BSA and SDS. pH 7.0, 50 mM phosphate buffer. SDS : C12E8 = 1 : 1 (molar ratio)

- : SDS
- : C12E8
- : SDS + C12E8

the fluorescent-labeled BSA has only one. It is considered that fluorescent-labeling of BSA induced the change in structure near the cysteine residue or blocking of the hydrophobic site where the alkyl chain of SDS adsors in the first step; thus, one of the patterns of high energy SDS binding disappeared.

The enthalpy change in the interaction between C12E8 and BSA was also exothermic (Fig.9). In the case of the hydrophobic interaction, the enthalpy change is usually endothermic because of the destruction of iceberg hydration. It is considered that the interaction between C12E8 and BSA is not a merely hydrophobic interaction but a polyoxyethylene chain-BSA interaction. When the molar ratio of BSA and C12E8 was more than 8, the adsorption enthalpy was nearly undetectable. This result indicates that less than 8 molecules of C12E8 can adsorb on a molecule of BSA. Concerning the interaction between BSA and AE, it is known that 4 molecules of C12E8 can adsorb on a molecule of BSA.

The adsorption enthalpy for the SDS-C12E8 mixed system, which was in the molar ratio of 1 : 1, was clearly different from the enthalpy for adsorption of either of the pure components. The adsorbing enthalpy was no longer detected at more than 15 moles of SDS in the mixed system per mole of BSA. This result reflects the adsorption of SDS which was as high as (15 molecules of SDS/molecule of BSA) in the SDS-C12E8 mixed system in the same ratio (Fig.5). The complicated pattern of the adsorbing enthalpy for the mixed system can be due to each surfactant being adsorbed not simply on BSA alone but in a complicated way such as the adsorption of C12E8 to the BSA denatured by SDS or the adsorption of C12E8 on SDS which was electrostatically bound to BSA by a tail-to-tail interaction, like admicelles. Considering that BSA was hardly denatured by the SDS/C12E8 mixed system, it is considered that the complicated pattern of the adsorbing enthalpy was mainly caused by the adsorption of C12E8 like admicelles.

At low SDS concentrations, SDS molecules bind specifically through ionic and hydrophobic interactions to BSA. This binding causes BSA to expand somewhat and allows noncooperative binding. Then there is an abrupt cooperative binding and formation of necklace and bead structures as the unfolding protein opens sites which induce micelle formation and the protein wraps around the micelles. It is not clear whether AE inhibits the specific binding of SDS to BSA. At least, AE inhibits the cooperative binding of SDS to BSA. It is considered that AE interacts with the hydrocarbon group of SDS which binds to BSA electrostatically, so that AE inhibits the formation of the necklace and bead structure between BSA and SDS.

4 Conclusions

The interaction between SDS and BSA in the presence or absence of AE was investigated. AE inhibited both the SDS binding to BSA and BSA denaturation. We consider that AE’s effect is due to the adsorption of AE on the alkyl chain of SDS which is electrostatically bound to BSA, like admicelles. The adsorption of AE causes the cooperative binding of SDS to BSA to notably decrease.

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References

水溶性切削油剤構成成分の製造と性状

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本稿では種々の原料から得られる水溶性切削油剤構成成分の合成法と性質について概説的に記述されている。これらの構成成分を分子中の官能基によって便宜的に下記のように分類し、著者の研究を中心に述べている。
(1) ハイドロキシル、(2) エステル、(3) 二塩基酸、(4) アルコール、(5) アミド、(6) 置換脂肪酸、(7) その他、(8) 試験法
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タンパク質－界面活性剤混合系の相互作用解析；
BSA-SDS/ポリオキシエチレンアルキルエーテル系

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界面活性剤混合系の牛血清アルブミン（BSA）変性作用を界面活性剤の吸着挙動を解析した。ポリオキシエチレンアルキルエーテル（アルコールエトキシレート：AE）は SDS の BSA への吸着、それに伴う変性を抑制した。いずれの抑制効果も AE の EO 鎖長、添加比率が大きいほど強かった。AE の電荷を有する表面への吸着は、SDS を混合することで飛躍的に増加した。SDS、AE の BSA への結合エネルギーは、混合系と単一系で明らかに異なった。以上の結果より、SDS による BSA の変性に対する AE の効果は、BSA に静電的に結合した SDS のアルキル鎖への AE の吸着によると考えられる。すなわち、AE が吸着することで BSA に静電的に結合した SDS のアルキル鎖を安定化し、BSA への SDS のさらなる結合を抑制するためと推察される。
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