Comparison of the Ability of Various Anionic Surfactants to Prevent Intramolecular Sulfhydryl Disulfide Exchange Reaction of Bovine Serum Albumin

Koichiro AOKI, Shigenori MAEZAWA, Tooru ITO, and Koichi HIRAMATSU

Department of Synthetic Chemistry, Faculty of Engineering, Gifu University (Kagamigahara, Gifu Pref.)

When the solution of bovine serum albumin (BSA) at pH 9 was heated at 65°C, new components (1'), (2), (3),......were formed. Here the components (1'), (2), and (3) are modified monomer, dimer, and probably trimer, respectively. The component (1') was formed by the intramolecular sulfhydryl disulfide exchange reaction. Components (2) and (3) were formed by the intermolecular sulfhydryl disulfide exchange reaction. If appropriate amounts of anionic surfactant were added to BSA solution, the formation of component (1'), or the intramolecular exchange reaction, was prevented. It is known since earlier data that fatty acid anions prevent the denaturation of BSA. The cause found in this study was that the anionic surfactant prevented the formation of component (1').

The minimum amounts of various surfactants to prevent the formation of component (1') were determined. The minimum molar ratio of surfactant/BSA was as follows: 5 for sodium tetradecyl sulfate and sodium palmitate, 6 for sodium tridecyl sulfate and sodium laurate, 7 for sodium dodecyl sulfate and sodium undecyl sulfate, 10 for sodium decyl sulfate, about 40 for sodium nonyl sulfate, and 28 for sodium hydrogen N-lauroyl-L-glutamate (LS-11). These values are conventional measures of a relative affinity of surfactant to BSA, indicating that the affinity of LS-11 is between that of sodium decyl sulfate and sodium nonyl sulfate. The present method is useful in determining the relative affinity of any anionic surfactant to BSA.

1 Introduction

The heat denaturation of bovine serum albumin (BSA) was studied previously by polyacrylamide gel electrophoresis. The BSA solution heat-treated in 0.1 M tris-EDTA-boric acid buffer at pH 9.0 gave several zones in the pattern of gel electrophoresis, and the percent composition of each component changed depending on the heating temperature and heating period. A photograph in ref. 1 or 2. Zones have been numbered (1), (1'), (2), (3),...... in the order of migration velocity. It was concluded that components in zones (1) and (1') were monomeric albumin, those in zones (2) and (3) are dimer and probably trimer, respectively. It was also concluded that the component (1') is a monomeric BSA modified by intramolecular SH - S-S exchange reaction. Components (2) and (3) were formed by intermolecular SH - S-S exchange reaction. The component (2') is a dimer which contaminates the commercial BSA, and is formed by some unknown bond other than S-S bond. The component (1'') (monomer) is formed only under certain limited conditions.

There are two kinds of denaturation, i.e., reversible and irreversible denaturations. This paper is concerned with the latter, irreversible heat denaturation.

After the BSA solution had been heated at 65°C, to which sodium dodecyl sulfate (SDS) was added (a molar ratio of SDS/BSA=12), the solution was analyzed by the polyacrylamide gel electrophoresis at room temperature. There was only zone (1), and no zones of (1'), (2), (3),......In other words, 12 SDS anions have an ability to prevent the exchange reaction of...
In this study the ability of various anionic surfactants to prevent the exchange reaction, or to prevent the formation of component (') of BSA at 65°C were compared. Larger the ability of the surfactant, lesser amount of it prevents the exchange reaction. Surfactants used were sodium alkyl sulfates having carbon number 9 to 14, sodium salts of fatty acids, and sodium hydrogen N-lauroyl-L-glutamate. Hereafter, sodium alkyl sulfate having carbon number n is denoted as SCnS.

2 Experimental

2.1 Surfactants

SCnS was synthesized from higher alcohol with carbon number n, which was gas-chromatographically pure, and chlorosulfuric acid. The reaction product was neutralized by sodium hydroxide and unreacted higher alcohol was removed by a Soxhlet extractor, and then crystallization was repeated.

Sodium palmitate and sodium laurate were purchased from Nakarai Chemical Co. These were purified by removing unreacted fatty acid by use of a Soxhlet extractor, and then were recrystallized.

Sodium hydrogen N-lauroyl-L-glutamate (LS-11) where R=C11H23, was supplied by Ajinomoto Co.

The cmc of SCnS was determined by the conductivity method on a TOA Model CM-1 DB conductometer. The cmc obtained in distilled water at 25°C were 2.08 mM for SC14S, 4.18 mM for SC13S, 8.23 mM for SC12S, 16.4 mM for SC11S, 31.2 mM for SC10S, and 63.6 mM for SC9S. These values are in agreement with those in the literature.

2.2 Bovine serum albumin

Armour crystallized BSA (lot no. M 72603) was used. The concentration of BSA was determined on a JASCO model UVIDEC-1 spectrophotometer, using the value E1\text{%}_279=6.67 at 279 nm. The molecular weight was assumed to be 66,000.

2.3 Heat treatment

The 0.1% BSA solution was prepared by use of 0.1M tris-EDTA-boric acid buffer at pH 9.1. A test tube, in which 2 ml of this solution was put, was sealed, and then heated at 65°C. The tube was dipped into ice-water after 30 min heating to stop the heat denaturation. The solution was then analyzed by disc gel electrophoresis. In order to observe the effect of surfactant, a series of mixtures of surfactant (D) and BSA (A), whose molar ratio (D/A) was changed systematically keeping the concentration of BSA constant, was prepared. Then the solutions were heat-denatured and analyzed electrophoretically in the same way as described above.

When alkyl sulfate or LS-11 was used, the BSA-surfactant solution was transparent both at room temperature and at 65°C. When sodium soap was used, there were small insoluble soap particles in the BSA-soap solution at room temperature. When this was heated at 65°C, it turned transparent. After the solution was cooled to room temperature, it was analyzed electrophoretically.

The pH was measured by a Hitachi-Horiba type F-5 ss pH meter.

2.4 Gel electrophoresis

The disc gel electrophoresis was conducted at pH 9.1 according to the standard procedure of Ornstein and Davis. A Mitumi model SJ-1050D electrophoresis apparatus and a Mitumi model SJ-1055A power supply were used. After the electrophoresis, the gel was stained by Amido black 10 B. Stained gels were scanned by a densitometer, Ozumer 82 of the Asuka Ind. Co.

2.5 Optical rotation

The optical rotation at 233 nm was measured using 0.2% BSA at various concentrations of SC12S and at 25±0.2°C. The phosphate buffer at pH 6.8 and ionic strength 0.1 was used. The apparatus was a JASCO model ORD/CD/UV-5 spectropolarimeter; the 1 mm cuvette was used.

2.6 Viscosity

The relative viscosity of the system BSA-SC12S was measured by using an Ostwald viscometer at 25±0.1°C. The phosphate buffer at pH 6.8 (ionic strength 0.1) was used. The sum of concentrations of BSA and SC12S was kept constant at 1.0%, only in this experiment.
3 Results

3.1 Minimum amounts of surfactants to prevent the formation of component (1')

Fig. -1 shows the effect of SC11S on the heat denaturation of BSA. Fig.-1 A is a pattern of native BSA. There are zones of (1) and (2'). Fig.-1 B is a pattern of BSA heat-denatured at 65°C for 30 min. Heat-treatment produced new zones of (1') and (2)*1. The molar ratio of D/A=1, 3 and 5 gave Figs.-1 C, 1 D and 1 E, respectively. Each figure showed a similar pattern and contained components (1'), (2), and (2'). Fig.-1 F had a small amount of component (1'), and Fig.-1 G and Fig.-1 H had almost none of it. These results are shown quantitatively in Fig.-2. It is shown that the component (1') diminishes at D/A=7. In other words, SC11S prevented the formation of (1') at D/A=7, or the minimum ratio of D/A, which prevented the formation of (1'), (D/A)\text{min}, for SC11S was 7 at 65°C. The sum of the components (2) and (2') is almost constant*2.

The value of (D/A)\text{min} was determined for each alkyl sulfate in the same way. The values are shown in Table-1. The same ability to prevent the formation of (1') was observed when the carbon number n is more than 11. The ability decreased with a decrease in carbon number.

The values of (D/A)\text{min} for sodium soaps and LS-11*3 are also shown in Table-1.

Table-1 Minimum amount of surfactant in mol per mol BSA, (D/A)\text{min}, to prevent the formation of component (1'). 65°C at pH 9.1.

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>(D/A)\text{min}</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC11S</td>
<td>5</td>
<td>Na palmitate</td>
</tr>
<tr>
<td>SC13S</td>
<td>6</td>
<td>Na laurate</td>
</tr>
<tr>
<td>SC15S</td>
<td>7</td>
<td>LS-11</td>
</tr>
<tr>
<td>SC1nS</td>
<td>10</td>
<td>GS-11</td>
</tr>
<tr>
<td>SC1S</td>
<td>Approx. 40</td>
<td></td>
</tr>
</tbody>
</table>

3.2 Optical rotation and viscosity

Fig.-3 shows a curve relating to the molar ratio of SC11S/BSA and specific rotation at 233 nm, \([\alpha]_{233}\). The curve has inflection points at D/A=10, 60, and 100. Fig.-4 shows a curve relating to weight ratio and relative viscosity. There is a minimum at point B, where the weight ratio of SC11S/BSA=95/5. There are

*1 The amount of component (3) is only trace in this experiment. This is because the heating period was 30 min. If heated longer, the amount of component (3) increases. Vague zone of component (1'') is observed between components (1) and (1') in Fig.-1 B.

*2 The reason for this is not known. There was the same trend, when other alkyl sulfates were used.

*3 A crude sample (GS-11), which was produced by use of fatty acids from coconut oil, was also used. This was not homogeneous, since the carbon number was widely different. Electrophoretic patterns for mixtures of BSA and GS-11 were not clear, having some tailing. Thus the definite value of (D/A)\text{min} was not determined. However, it seemed to be far greater than 28.
inflection points at C, D, and E, where the weight ratios are 67/33, 55/45, and 45/55, respectively.

4 Discussion

There are components (1') and (2) in Fig. 1 B. These are irreversibly denatured components, since these components are produced during the course of heat-treatment, and are stable even after keeping those at room temperature. The occurrence of the SH – S-S exchange reaction is one of the aspects of irreversible denaturation.

4.1 Characteristics of component (1')

First of all, properties of component (1') are compared with those of the native BSA. Isolation of component (1') in the pure state by a preparative disc gel electrophoresis and its properties have been studied. The extinction coefficient $E_{1%279}^{1%210}$ for component (1') was 6.6. Since the value for native BSA in distilled water is 6.67, there is no apparent difference between them. However, there are several indications that conformation of (1') differs slightly from that of native BSA. (a) Sedimentation coefficient of (1') is smaller than that of native BSA. Values of $S_{2%w}$ was 3.5 and 3.6 for component (1') and native BSA, respectively. (b) The helical content of (1') is smaller than the normal value. $\beta_0$ was 42% and 51% for component (1') and native BSA, respectively. (c) Native BSA enhanced the absorbance of the dye 2-(4-hydroxyphenylazo) benzoic acid near 480 nm at pH 7.0. Component (1') did not enhance that at all. (d) The difference spectrum of bromocresol green induced by native BSA had a trough at 616 nm and a peak at about 668 nm. Those induced by component (1') had a trough at 610 nm and a peak at 655 nm. (e) The isoelectric points determined by the technique of isoelectric focusing was pH 5.9 and 4.9 for component (1') and native BSA, respectively.

4.2 Outline of the interaction of BSA and detergent

The interaction was studied most extensively on the system BSA–SC$_{12}$S. There are vast number of data on this system. Brief survey of the interaction is given below. The Tiselius electrophoresis and other techniques have revealed that the first stoichiometric complex is AD$_{11}$ (A : BSA, D : SC$_{12}$S). Some workers state that it is AD$_{10}$, and some state it is AD$_{12}$. In this statistical binding region, the composition of the complex increases continuously from AD$_0$ to AD$_{12}$. The second and the third stoichiometric complexes are AD$_{11}$ and AD$_{11}$. In the earlier date, the value of $n$ was believed to be 55. Later it was determined to be 38 for the system of BSA and sodium

*4 The helix content determined by the Moffitt–Yang equation.
dodecylbenzenesulfonate[13]. In the cooperative binding region, the composition of the complex jumps from 12 to n, and from n to 2n.

Three inflection points on the curve in Fig.-3 indicated that the helix content[17] decreased discontinuously when each stoichiometric complex was formed. The relative viscosity is the minimum at the weight ratio of SC18S/BSA= 95/5. The composition of the complex at this point is AD12. This indicates that the BSA is most compact and stable when 12 SC18 anions are bound. The point C corresponds to the complex AD12. There is no inflection point at ADn.

It is well known that detergent anions are bound to BSA through both hydrophobic and electrostatic forces in the statistical binding region. Direct evidence for this binding mechanism was obtained by Oakes[19] through the study by use of NMR technique.

The study of the interaction of BSA with the detergents having carbon number other than 12 is only a few.

4.3 Prevention of the exchange reaction by detergents

Table-1 shows that a slight amount of SC18S, SC19S, SC20S, or SC21S prevents the intramolecular exchange reaction in BSA at 65°C. More exactly, 5 to 7 mols of these detergents per mol of BSA prevented the exchange reaction. Greater amount of SC22S was needed to prevent the same reaction.

In the statistical binding region, the stability of BSA increased with the increase in the number of anionic ligands bound[19]. Increased resistance against proteolytic digestion of serum albumin in the presence of anionic detergents was reported[19]. Another evidence may be that the relative viscosity decreases with the increase in the amount of SCnS bound, i.e., it decreases from point A to B in Fig.-4. This suggests that the definite number of detergents stabilize or tighten the BSA enough to prevent the exchange reaction. Since the affinity of SC18S-SC21S to BSA is high, or the binding constant, K, of the system of each detergent and BSA is large (see below), almost all the detergents used are bound to BSA. Since the affinity of SC22S to BSA is lower than that of SC21S[21], greater amount of SC22S is needed to prevent the exchange reaction. In other words, the difference in the value (D/A)min is a reflection of the difference in the affinity of the detergent to BSA.

4.4 Values of (D/A)min for soaps and LS-11

The values of (D/A)min for two soap anions are 5 and 6. These are the same as the values for SC18S and SC19S. This is reasonable, because the binding constants K are the same for two soaps and SC21S. The K was determined for a series of alkyl sulfates at 2°C. The values for high-energy binding sites of SC18S, SC19S and SC20S are in the order of 107[22]. The K for laurate is in the order of 105[22]. Since the K at 65°C is not known, it is assumed that the temperature coefficients of K are the same for soap and alkyl sulfate.

The value of (D/A)min for LS-11 was greater than that for SC18S or SC19S, although R in LS-11 was C17H33. A reason may be that LS-11 has two negative charges. There is no binding isotherm of LS-11. However, the present conventional method revealed that the affinity of LS-11 to BSA was between that of SC20S and SC21S.

4.5 Effect of fatty acid contaminating BSA

Commercial BSA has a slight amount of fatty acid as a contaminant. Fatty acid content of Armour crystallized BSA used in our laboratory was 0.90 mol per mol BSA[11]. The undefatted BSA was used in this study, under the observation for the prevention of the exchange reaction. This suggests that fatty acid as a contaminant had no serious effect on the exchange reaction. If defatted BSA was used, the value of (D/A)min would be larger by 1 than that obtained in the present study.

4.6 Concluding remark

The minimum amount of each surfactant to prevent the intramolecular exchange reaction, or the value of (D/A)min, is a conventional measure of a relative affinity of surfactant to BSA at 65°C. Although the binding isotherm gives thermodynamic data, accurate determination of the isotherm takes a long time. If the prompt determination of approximate value of the affinity is needed, the present method is available.
It is well known since earlier date that fatty acid anion or detergent anion prevents the urea denaturation of BSA. It has been found that the SH-S-S exchange reaction is one of the aspects of urea denaturation as well as heat denaturation. The prevention of the formation of component (1') by appropriate amounts of anionic surfactants is the prevention of these denaturations themselves, since it is known that the components (2), (3),..., are formed by the polymerization of component (1').

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References