Interaction between Surface Active Agents and Proteins. II. Electrophoretic Investigation of the System Sodium Dodecyl Sulfate and Egg Albumin

By Koichiro AOKI*

(Received August 12, 1955)

Introduction

Precipitation occurs when the anionic detergent and the protein are mixed at a lower pH than that of the isoelectric point of the protein, and no precipitation occurs when they are mixed at a higher pH than that of the isoelectric point of the protein. In the latter case a transparent solution is obtained, so we cannot confirm whether or not they react, merely from the appearance of the solution when mixed. It is known by the electrophoretic investigation, however, that the complex is formed in the solution. Lundgren et al.[1] proved the existence of the complex in the system alkylbenzenesulfonate-egg albumin, and Putnam and Neurath[2] proved the existence of the complex in the system sodium dodecyl sulfate-horse serum albumin. Further, Yang and Foster[3] studied the interaction between sodium dodecylbenzenesulfonate and egg albumin, and also between this detergent and bovine serum albumin by both the electrophoretic method and the dialysis equilibrium method, holding a different opinion on the behavoir of serum albumin toward detergent from that of Putnam and Neurath.

We discussed in Part I[4] the precipitation reaction which occurred when sodium dodecyl sulfate and egg albumin were mixed at a lower pH than that of the isoelectric point of the protein. No electrophoretic investigation has yet been made on the interaction between sodium dodecyl sulfate (SDS) and egg albumin on the alkaline side of the isoelectric point of the protein. For this reason we investigated this interaction electrophoretically at various pH's and the results obtained are discussed.

Experimental

SDS and egg albumin used were prepared by the same method as described in Part I.

We used Hitachi's electrophoretic apparatus equipped with the Schlieren diagonal system. The size of the cell for electrophoresis is $2\times15\times40$ mm. All experiments were carried out in a thermostat at 25 ± 0.01°C.

We prepared and electrophoretically analyzed a series of samples, the weight mixing ratio of which varied, while the sum of the concentration of SDS and that of egg albumin was kept constant at 1.0%. In the same way an electrophoretic study was made on samples the total concentration of which was 0.5%. The ionic strength (μ) of all the buffer solutions used was 0.1. The value of pH and composition of buffer solutions are as follows. No dialysis of the sample was

pH	Composition
5.4	sodium acetate and hydrochloric acid
6.8	sodium monohydrogenphosphate and sodium dihydrogenphosphate
7.6	〃 〃
9.6	〃 〃
9.9	sodium carbonate and sodium bicarbonate
10.8	〃 〃 〃 〃

carried out before electrophoresis, because we found that a small quantity of SDS was dialysed through the membrane into the outer solution and that the composition of egg albumin to SDS was slightly changed during the dialysis.

Results

Fig. 1 shows typical electrophoretic patterns. Fig. 2 shows the mobilities of components calculated from the distance of migration on the descending side. We can decide from this figure that the two components existing in the region of albumin excess are egg albumin and complex, and that the two components in the region of SDS excess are complex and SDS.

We can calculate the number of positive and negative charges on one egg albumin molecule. Curve A in Fig. 4 shows the numbers of positive charges on one egg albumin molecule at each pH.

* Present temporary address: Department of chemistry, Purdue University, Lafayette, Indiana, U.S.A.

1) H.P. Lundgren, D.W. Elam and R. A. O'Connell, *J. Biol. Chem.*, **149**, 183 (1943); H.P. Lundgren and R.A. O'Connell, *Ind. Eng. Chem. Ind. Ed.*, **36**, 370 (1944).

2) F.W. Putnam and H. Neurath, *J. Biol. Chem.*, **159**, 195 (1945).

3) J.T. Yang and J.F. Foster, *J. Am. Chem. Soc.*, **75**, 5560 (1953).

4) K. Aoki and J. Hori, This bulletin, **29**, 104 (1956).

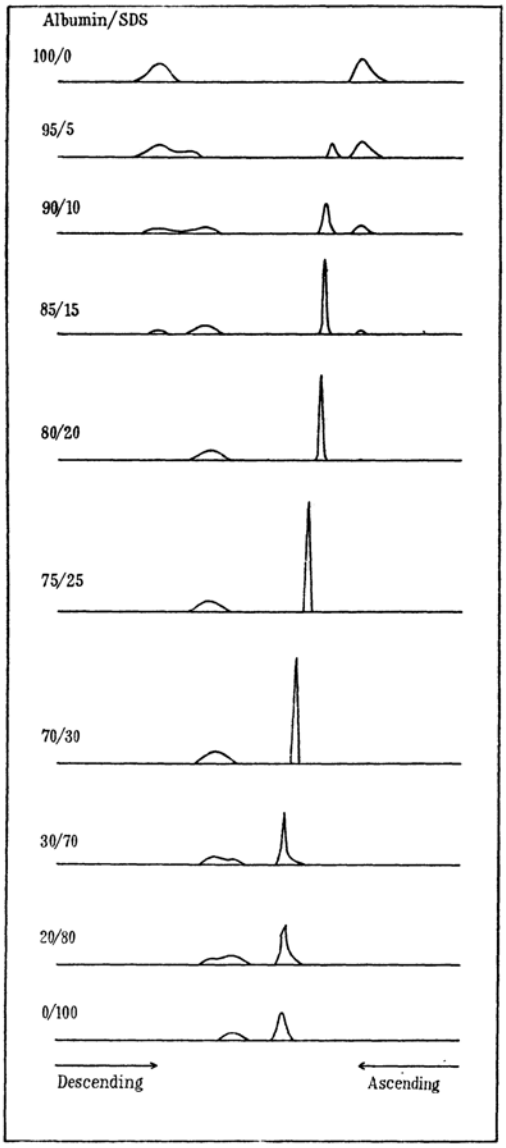

Fig. 1. Electrophoretic patterns of the system sodium dodecyl sulfate-egg albumin at pH 6.8 and ionic strength 0.1. Total concentration is 1.0%.

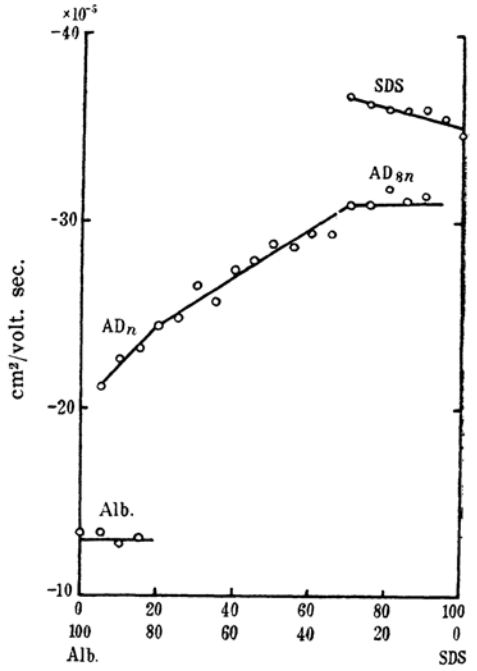

Fig. 2. Electrophoretic mobilities of components in the sodium dodecyl sulfate-egg albumin system.

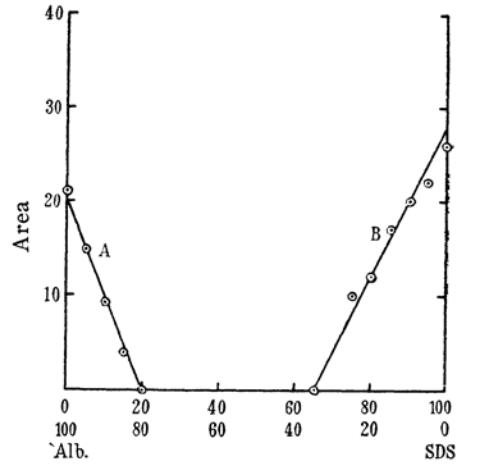

Fig. 3. A: Relation between the area of egg albumin boundary (in arbitrary unit) and the mixing ratio. B: Relation between the area of SDS boundary (in arbitrary unit) and the mixing ratio.

Discussion

Electrophoretic Patterns at pH 6.8.—Electrophoretic patterns obtained can be divided into the following three groups.

The first group comprises the patterns obtained when the mixing ratio albumin/SDS is between 100/0 and 80/20, each pattern having two components, i.e., albumin and complex. With the change of the mixing ratio albumin/SDS, the area of the complex boundary in the pattern increases and that of egg albumin boundary decreases. The electrophoretic pattern on the mixture of albumin/SDS ratio of 80/20 shows merely a trace of the albumin boundary. In Fig. 3, the relation between the composition and the area of albumin boundary is shown. From the slope of the straight line A and the value of 46000 and 288 for the molecular weight of egg albumin and SDS respectively, we can calculate and confirm the fact that about 40 molecules of SDS are bound to one

molecule of egg albumin. In other words, when the mixing ratio albumin/SDS is between 100/0 and 80/20, the composition of the complex is constant and has the formula AD_{40} (A: albumin, D: detergent). We can find that 46 detergent molecules are bound to one egg albumin molecule at pH 6.5, if we use the data given by Lundgren et al. for the electrophoretic experiment on the system alkylbenzenesulfonate-egg albumin[1]. Yang and Foster[3] studied the interaction between sodium dodecylbenzenesulfonate and egg albumin, and found that 43 detergent molecules were bound to one egg albumin molecule at pH 7.6. Further, as described before, we found that about 40 molecules of SDS were bound to one egg albumin molecule at pH 4.2, a lower pH than that of the isoelectric point of egg albumin. Thus, the number of detergent molecules which are bound to one egg albumin molecule seems to be nearly the same for all cases.

As seen in curve A in Fig. 4, the number of positive charges of one egg albumin molecule at pH 6.8 is 38. We found that the composition of the complex first formed was AD_{40}, and this number 40 is almost equal to the number of positive charges on one egg albumin molecule at pH 6.8. Therefore we can assume that the complex is formed through the electrostatic attraction between one negative charge of dodecyl sulfate ion and one of the positive charges on egg albumin molecule.

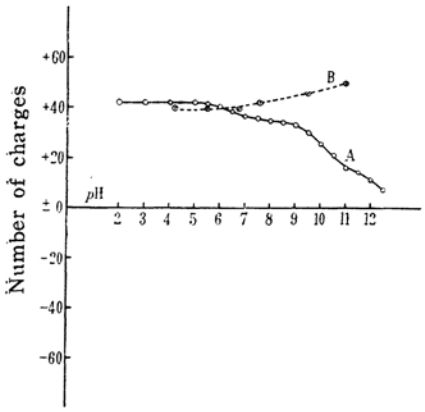

Fig. 4. A: Number of positive charges on one egg albumin molecule at each pH. B: Number of SDS bound to one molecule of egg albumin.

The second group of patterns are obtained when the mixing ratio albumin/SDS is between 80/20 and 35/65. All patterns of this group have only one complex. The mobility of the complex changes continuously with the mixing ratio, so we can assume that the composition of this complex changes continuously with the mixing ratio. As pointed out before, the composition of the complex at the point albumin/SDS=80/20 is AD_n (n=40). The composition of the complex formed at the point albumin/SDS=35/65 is AD_{8n}. It is our opinion that the composition of the complex changes continuously after the formation of AD_n to the completion of AD_{8n}.

The thired group of patterns are obtained when the mixing ratio albumin/SDS is between 35/65 and 0/100. In this region, the patterns have two components, i. e., complex and SDS, and the mobilities of both components are constant, independent of the mixing ratio. Thus, in the region of SDS excess, the mobility of the complex is constant and an unreacted SDS boundary appears. This means that the range of composition of the complex is limited. The line B in Fig. 3 shows the relation between the area of SDS boundary and the mixing ratio. The composition of the complex coexisting with unreacted SDS is found to be AD_{8n} in the same manner as described in the preceding section.

It may be added that our division of the electrophoretic patterns of the syetem SDS-egg albumin is not the same as that presented by Putnam and Neurath for the system SDS-horse serum albumin.

In the case of solutions in which the total concentration was 0.5%, the electrophoretic results were almost the same as those obtained with solutions of total concentration 1.0%.

Curve A in Fig. 5 shows the relation between the relative viscosity (value compared

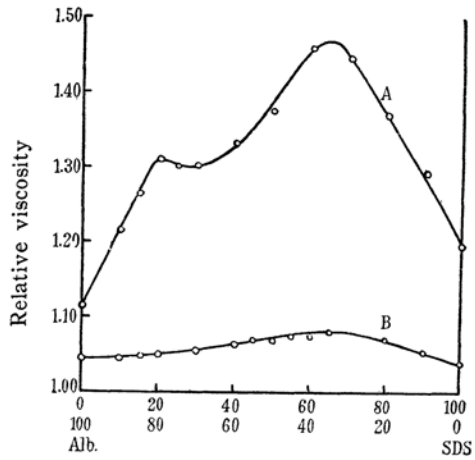

Fig. 5. Relative viscosity, compared to the buffer solution, of the system egg albumin-SDS at pH 6.8 and ionic strength 0.1. A: Total concentration 4.0%. B: Total concentration 1.0%. 25±0.01°C.

with that of the buffer solution) and the mixing ratio, the total concentration being kept at 4.0%. We can see that there are two maxima at points where albumin/SDS=80/20 and 35/65.

The compositions at these maxima are equal to those which divide the electrophoretic results into three groups.

Electrophoretic Patterns at Various pH's.—Similar experiments as above were carried out at a total concentration 1.0%, and at pH 5.4, 7.6, 9.6, 9.9 and 10.8.

At each pH the electrophoretic patterns change with the mixing ratio in a similar manner as when pH is 6.8, but in the region of albumin excess, there is a slight change in values of the mixing ratio at which the albumin boundary disappears. The number of SDS molecule bound to one molecule of egg albumin at this mixing ratio increases from 40 to about 50 with the increase of pH. Curve B in Fig. 4 shows the relation between pH and the number of bound SDS to one egg albumin molecule.

Comparing curves A and B in Fig. 4, we can see that below pH 7 the number of positive charges of one egg albumin is almost equal to the number of SDS molecules which are bound to one egg albumin molecule. In this region it is possible to assume that one positive charge on egg albumin and one negative detergent ion combine electrostatically as described above, but when pH is above 7 this relation cannot hold. The cause of this is not made clear in this study alone. In the experiment at different pH from 6.8, in the region of SDS excess, SDS boundary disappears when the mixing ratio albumin/SDS is between 35/65 and 30/70.

Mechanism of the Combination of Egg Albumin and SDS.—Now we compare the next two electrophoretic patterns with each other: the one obtained on the sample pH 6.8, μ 0.1, albumin/SDS=95/5 and the total concentration 1.0%, and the other pH 6.8, μ 0.1, albumin/SDS=95/5 and the total concentration 0.5%. We can find that the shapes of the patterns are identical, although the areas covered by the corresponding boundaries are unequal. In addition, as seen in Fig. 5a in Part I[4], the CMC of SDS is 0.04% (0.0013 mol./1.) when the ionic strength is 0.1, so that in the first sample the micellar SDS exists, but in the second sample SDS exists as a single ion not forming a micelle. Considering these facts we can find in the reaction between egg albumin and SDS that it is not reasonable to assume that SDS reacts only in a micellar form, and that it is reasonable to assume that a single ion, not forming a micelle, can react.

Summary

We studied electrophoretically the interaction of SDS and egg albumin in the pH range between 5.4 and 10.8, higher pH's than that of the isoelectric point of egg albumin. Results obtained are as follows.

(1) The electrophoretic patterns of the system egg albumin-SDS can be divided into three groups. This method of division is different from that in the system SDS-horse serum albumin by Putnam and Neurath. The first group of the division contains the patterns obtained when the mixing ratio albumin/SDS is between 100/0 and 80/20, and here the complex AD_n (n=40, A: albumin, D: detergent) and egg albumin coexist. The second group contains the patterns obtained when the mixing ratio albumin/SDS is between 80/20 and 35/65 and in this region the composition of the complex changes continuously from AD_n to AD_{8n}. The third region appears when the mixing ratio albumin/SDS is between 35/65 and 0/100 and here the complex AD_{8n} and SDS coexist. The above mixing ratio values are obtained at pH 6.8, and they change somewhat as a function of pH.

(2) Below pH 7, a number n of the first complex AD_n equals the number of positive charges on one egg albumin, but above pH 7, this relation cannot hold.

(3) In the reaction between egg albumin and SDS, it is not assumed that SDS reacts only in a micellar form but that SDS existing as a single ion can react.

The author is sincerely greateful to Professor Rempei Goto of the Institute for Chemical Research, Kyoto University for his kind guidance during this study.

Chemical Laboratory, Nagoya City University, Nagoya