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Interactions of Horse Serum Albumin with Anionic and Cationic Detergents

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Interactions of horse serum albumin (HSA) with sodium dodecyl sulfate (SDS) and dodecylpyridinium bromide (DPB) were studied mainly by electrophoresis in the *p*H region between 3.2 and 10.6. The electrophoretic study was made using SDS when the *p*H was on the alkaline side of the isoelectric point and using DPB when it was on the acid side. The mode of interaction changed discontinuously at several *p*H values. The changes in the pattern in the region of weight mixing ratio (HSA/detergent) between 95/5 and 70/30 was as follows. At *p*H 5.6 and *p*H 6.8 there were, besides AD_{12}, two discrete complexes which are known to be AD_n and AD_{2n} (A:HSA, D:SDS, and $n = 105/2$). At *p*H 8.9 there was only one discrete complex besides AD_{12}. At *p*H 10.6 and *p*H 3.2 there was only a single boundary. At *p*H 4.4 DPB converted the N form of serum albumin into F form. It is considered that this change in electrophoretic pattern with *p*H reflects changes in the internal structure of serum albumin, changes associated with *p*H which have been recognized recently. It is known that serum albumin expands below *p*H 3.5 and undergoes an isomerization reaction between *p*H 3.5 and 4.5. It is also known that there is a subtle change in serum albumin when the *p*H is above 7.5 and that some abnormality occurs when it is above 10. Results by electrophoresis and precipitation curve showed that the complex, having a composition between AD_1 and AD_{12}, existed on both the acid and alkaline sides of the isoelectric point. Another result showed that HSA was saturated with $6n$ SDS ions in the *p*H range 3.5—10.6.

Introduction

Many studies have been done on the interaction between surface active agents and proteins. Most of the quantitative studies were done on the interaction of an anionic detergent with serum albumin. Since the pioneer work by Putnam and Neurath,[1-3] studies were made on the stoichiometric property of the binding of detergent anion to serum albumin in a limited *p*H range.[4-8] Almost all the results of these studies are consistent. Equilibrium dialysis was used[4-6] to determine accurately the number of detergent ions bound to serum albumin.

Previously the author studied the interaction between egg albumin and sodium dodecyl sulfate (SDS) and found some differences between the reactivity of egg albumin and that of serum albumin toward SDS.[9,10] The interaction between SDS and horse serum albumin (HSA) at various *p*H values, *i.e.*, between 3.2 and 10.6, not only in the region of protein excess but also in the region of detergent excess has now been studied. The interaction between HSA and cationic detergent, dodecylpyridinium bromide (DPB), also was studied as a function of *p*H, since only a few studies on this interaction have been made.[11]

Experimental

HSA and SDS were prepared as previously.[9] DPB was synthesized from dodecyl bromide and pyridine.[12] The melting point was 155°. By measuring the specific conductivity at 25.00°, the critical micelle concentration in distilled water was found to be 0.40% (0.012 mole/l.).

The electrophoresis was made at various *p*H values and at 25 ± 0.01° in a Tiselius-type apparatus, Model HT-B of the Hitachi Ltd., Tokyo, equipped with the schlieren diagonal system. The experiment was conducted as previously.[10] The total concentration, the sum of the concentrations of HSA and SDS, was kept constant at 1.0%. All runs were made at the ionic strength 0.1. Mixtures of solutions of HSA and SDS were analyzed after allowing them to stand overnight at room temperature. Some supernatants were analyzed electrophoretically when precipitate appeared.

When precipitate was formed, the percentage of HSA which had precipitated was determined as a function of mixing ratio. Assuming the percentage of nitrogen in HSA to be 16.0% and determining the concentration of nitrogen by the micro-Kjeldahl analysis, the precipitation percentage was determined. By plotting the percentage value against the mixing ratio, a precipitation curve[9] was obtained. The analysis of nitrogen was made 72 hours after mixing solutions of HSA and SDS.

The interaction between HSA and DPB was studied in the same way as described above.

The following buffer solutions were used: *p*H 3.2, glycine, hydrochloric acid and sodium chloride; *p*H 3.5 and 4.4, hydrochloric acid and sodium acetate; *p*H 5.6, acetic acid and sodium acetate; *p*H 6.8, sodium monohydrogen phosphate and sodium dihydrogen phosphate; *p*H 8.0, sodium dihydrogen phosphate and sodium tetraborate; *p*H 8.9 and 10.6, sodium carbonate and sodium bicarbonate.

Results

HSA–SDS.—Typical electrophoretic patterns at *p*H 6.8 are shown in Fig. 1. Patterns were usually enantiographic. Mobilities calculated on the descending side are shown in Fig. 2 *versus* the weight mixing ratio of HSA to SDS (HSA/SDS). When HSA/SDS was between 80/20 and 70/30, where patterns were less enantiographic, the mean of the mobilities of the ascending and descending patterns was plotted in the figure. Figs. 1 and 2 show that the electrophoretic pattern at *p*H 6.8 can be divided into six groups as shown in Table I. Of course these boundary mixing ratio values are approximate. When HSA/SDS was between 95/5 and 80/20, the area of the faster one of the two moving boundaries increased with the amount of SDS at the expense of the slower boundary. When the mixing ratio value was lower than 80/20, a new boundary

TABLE I

DIVISION OF ELECTROPHORETIC PATTERNS

Region of mixing ratio (HSA/SDS)	No. of boundaries *p*H 5.6 and 6.8	*p*H 8.9
100/0—95/5	1	1
95/5—80/20	2	2
80/20—70/30	2	
70/30—45/55	1	1
45/55—5/95	2	2
0/100	1	1

(1) F. W. Putnam and H. Neurath, THIS JOURNAL, **66**, 692 (1944).
(2) F. W. Putnam and H. Neurath, *J. Biol. Chem.*, **159**, 195 (1945).
(3) F. W. Putnam and H. Neurath, *ibid.*, **160**, 397 (1945).
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(5) J. T. Yang and J. F. Foster, *ibid.*, **75**, 5560 (1953).
(6) M. J. Pallansch and D. R. Briggs, *ibid.*, **76**, 1396 (1954).
(7) B. S. Harrap and J. H. Schulman, *Disc. Faraday Soc.*, **13**, 197 (1953).
(8) G. Markus and F. Karush, THIS JOURNAL, **79**, 3264 (1957).
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(10) K. Aoki, *ibid.*, **29**, 369 (1956).
(11) J. F. Foster and J. T. Yang, THIS JOURNAL, **76**, 1015 (1954).
(12) F. Tanaka, N. Inoue and Y. Namba, *J. Pharm. Soc. Japan*, **63**, 353 (1943).

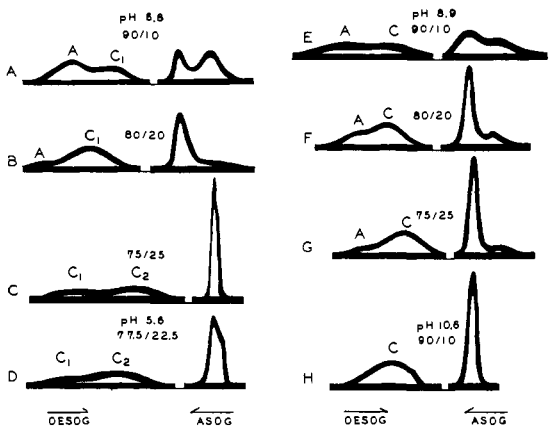

Fig. 1.—Typical electrophoretic patterns of system HSA–SDS. Ionic strength 0.1, total concentration 1.0%.

	*p*H	HSA/SDS	Ma.	Sec.
A	6.8	90/10	5.0	3,600
B	6.8	80/20	5.0	3,600
C	6.8	75/25	5.0	5,400
D	5.6	77.5/22.5	3.5	8,400
E	8.9	90/10	3.5	10,800
F	8.9	80/20	3.5	3,600
G	8.9	75/25	3.5	3,600
H	10.6	90/10	3.5	2,700

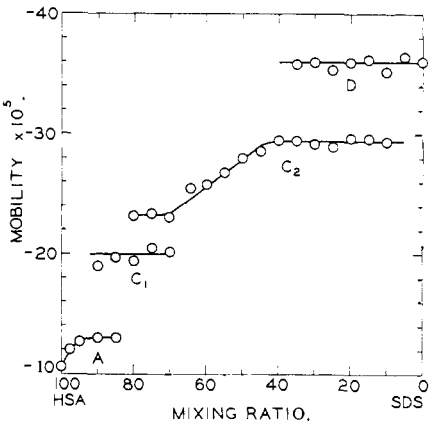

Fig. 2.—Mobilities of components of system HSA–SDS at *p*H 6.8. Abscissa is weight mixing ratio (HSA/SDS) and ordinate is mobility in unit cm.²/volt. sec. Mobilities were calculated on descending pattern. A, HSA and complex from AD_1 to AD_{12}; C_1, AD_n; C_2, complex from AD_{2n} to AD_{6n}; and D, SDS.

appeared. The area of the new boundary increased with increase of the amount of SDS, and at HSA/SDS = 70/30 the pattern had a single boundary. In the region of mixing ratio between 45/55 and 5/95, again there were two boundaries.

Typical electrophoretic patterns at *p*H 8.9 and 10.6 are shown in Fig. 1. It was found that at *p*H 5.6 electrophoretic patterns could be divided in the same way as at *p*H 6.8, but patterns at *p*H 8.9 had to be divided in a different way. While patterns obtained in the region of mixing ratio between 95/5 and 70/30 were divided into two groups at *p*H 5.6 and 6.8, patterns at *p*H 8.9 changed continuously in one step. The straight line CD in Fig. 4a shows that the area of the slower moving boundary at *p*H 8.9 decreased linearly with the weight mixing ratio. The area was measured on the descending pattern. Mobilities at *p*H 8.9 are given in Fig. 3a. At *p*H 10.6 patterns were quite different. There was no discrete complex. (Strictly speaking, there was a small amount of faster moving component on the descending pattern of HSA and on that obtained when albumin was in excess.) As is seen in Fig. 3b, the mobility of the component increased linearly with the amount of SDS.

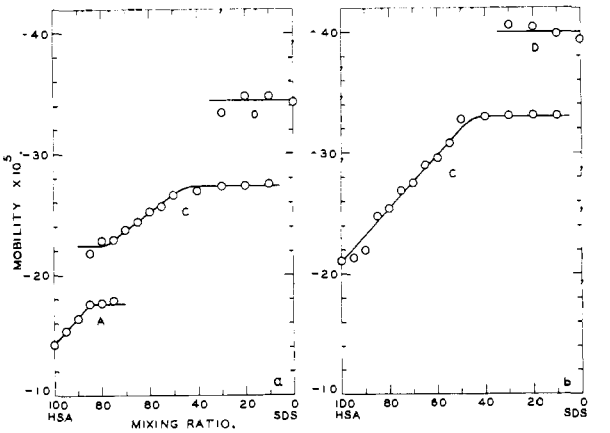

Fig. 3a.—Mobilities of components of system HSA–SDS at *p*H 8.9: A, HSA and complex from AD_1 to AD_{12}; C, complex from AD_{2n} to AD_{6n}; D, SDS.

Fig. 3b.—Mobilities of components of system HSA–SDS at *p*H 10.6: C, complex up to AD_{6n}; D, SDS.

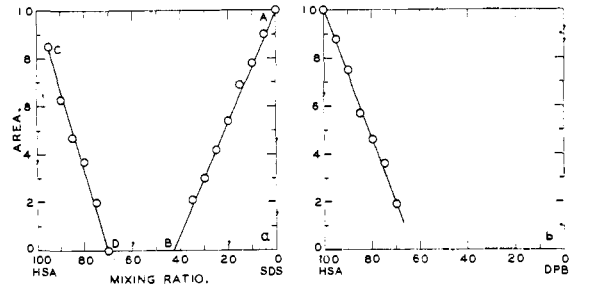

Fig. 4a.—Straight line AB indicates relation between area of SDS boundary and weight mixing ratio of system HSA–SDS. Intersecting point B and slope of line AB are independent of *p*H between 3.5 and 10.6. Straight line CD indicates relation between area of slower boundary (AD_{12}) and weight mixing ratio of system HSA–SDS at *p*H 8.9.

Fig. 4b.—Sum of areas of N and F_1 boundaries *versus* mixing ratio of system HSA–DPB at *p*H 4.4. Area was measured on descending pattern in arbitrary unit.

Precipitation curves at *p*H 3.5 and 4.2 are shown in Fig. 5. (The curve at *p*H 4.2 has been shown in Fig. 7 of ref. 9.) Plots at these two different *p*H values fall on a common curve when albumin is in excess. Supernatants or transparent solutions at *p*H 3.5 migrated toward the cathode when HSA/SDS was between 100/0 and 85/15 and moved toward the anode when it was between 60/40 and 0/100.[13] Although the sign of the complex changed according to the weight mixing ratio,

(13) When the weight mixing ratio was between 80/20 and 65/35, the boundary was not clear because of the low concentration of the complex in supernatants.

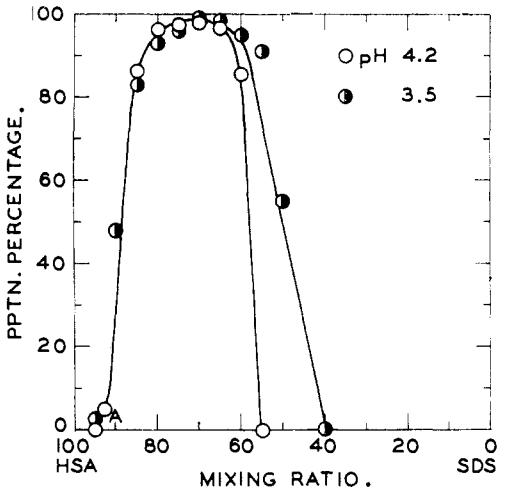

Fig. 5.—Percentage of HSA turned into precipitate *versus* mixing ratio of system HSA–SDS. Ionic strength 0.34. Total concentration was 1.0% at *p*H 3.5 and 2.0% at *p*H 4.2.

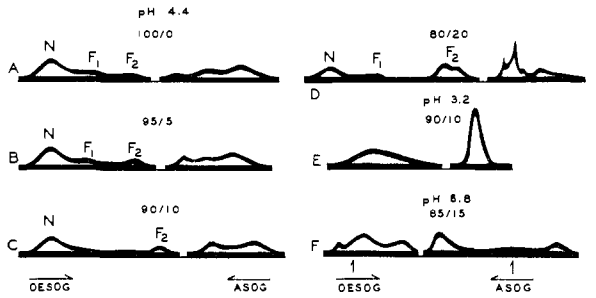

Fig. 6.—Typical electrophoretic patterns of system HSA–DPB. Ionic strength 0.1, total concentration 1.0%. Current 5.0 mamp. Vertical arrows indicate position of initial boundaries. Ascending direction is toward left and descending toward right.

	*p*H	HSA/DPB	Sec.
A	4.4	100/0	3600
B	4.4	95/5	3600
C	4.4	90/10	3600
D	4.4	80/20	3600
E	3.2	90/10	4800
F	6.8	85/15	2400

the boundary of free SDS appeared at the same weight mixing ratio value as at the alkaline *p*H.

HSA–DPB.—Although the electrophoresis of this system was carried out under the same conditions as in the study of the system HSA–SDS, patterns were less enantiographic when compared with those of the latter system. Resolution of boundaries was better on descending than on ascending.[14] Typical electrophoretic patterns are shown in Fig. 6. At *p*H 4.4 there were three boundaries. This is in agreement with the result obtained previously, *i.e.*, bovine plasma albumin (BPA) resolves into three components at *p*H between 3.5 and 4.5 and at ionic strength 0.1.[15] These three boundaries were termed N, F_1 and F_2, since it was assumed that, when three boundaries existed, the two faster moving boundaries were F form. The straight line in Fig. 4b indicates that the sum of areas of N and F_1 boundaries decreased linearly with the mixing ratio in the region between HSA/DPB = 100/0 and 70/30. The area was measured on the descending pattern. The F_2 boundary separated into two boundaries when the content of DPB was high. Mobilities are shown in Fig. 7. At *p*H 3.2 only a single boundary was obtained in the region of weight mixing ratio between 90/10 and 60/40. (There was a trace of faster component on the descending pattern when the content of DPB was high.) Foster and Yang[11] also obtained a single boundary in a study of interaction between BPA and cationic detergent at *p*H 3.3.

Unexpectedly, there was no precipitate at *p*H 6.8 and ionic strength 0.1, but a small amount of precipitate was formed instantly at ionic strength 0.02. Precipitation curves determined at ionic strength 0.02 and at various *p*H values indicated that the amount of precipitate increased with increase of *p*H.[16] Thus it is noted that the lower the

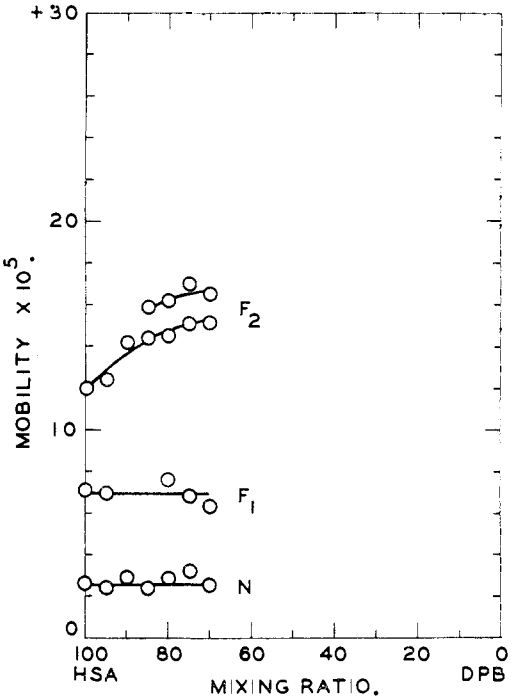

Fig. 7.—Mobilities of components of system HSA–DPB at *p*H 4.4. Because of isomerization, there were three boundaries, N, F_1 and F_2.

(14) It is thought that the pattern was more disturbed by the presence of free unbound cationic detergent. It is seen that the affinity of bovine plasma albumin to cationic detergent is lower than that to anionic detergent when data of equilibrium dialysis are compared. It is calculated from the table of ref. 5 that 85–81% of sodium dodecylbenzenesulfonate ions were bound to bovine plasma albumin at *p*H 7.7, as described in the text. It is also calculated from the table of ref. 11 that 40–50% of dodecyldimethylbenzylammonium chloride ions were bound to bovine plasma albumin at *p*H 3.3. Although these percentage values cannot be applied directly to the present system, it is qualitatively known that more anionic detergents than cationic detergents are bound to serum albumin.

(15) K. Aoki and J. F. Foster, THIS JOURNAL, **79**, 3385 (1957); see also, J. R. Cann and R. A. Phelps, *ibid.*, **79**, 4672 (1957).

(16) When the amount of precipitate was small, the percentage values were sometimes negative. Control runs showed that DPB solution was decomposed by 20% in the same procedure of Kjeldahl analysis as applied to HSA solution. It is thought that the average percentage of decomposition of nitrogen in supernatant differed a little in the presence of DPB. Hence these curves have only a qualitative meaning. Maxima of the precipitation percentage were: at *p*H 6.8, 4% at HSA/DPB = 87.5/12.5; at *p*H 8.9, 21% at HSA/DPB = 80/20; at *p*H 10.6, 85% at HSA/DPB = 65/35.

ionic strength and the higher the *p*H, the more precipitate appears. It is added that there was no precipitate when the amount of HSA was in great excess in the *p*H region studied.

Therefore the electrophoretic study was made at *p*H 6.8 and 8.0 and at ionic strength 0.1. One of the patterns is shown in Fig. 6. At *p*H 6.8, there was a single boundary when the weight mixing ratio was between 100/0 and 95/5, and there were two or three boundaries between 90/10 and 60/40. In the latter region of mixing ratio, electrophoretic mobilities of complexes increased with increase of the amount of DPB. Since the HSA is negatively charged at this *p*H and DPB positively charged, the complex having net charge zero appeared when the weight mixing ratio was proper. Because of the convection due to cationic detergent, the exact value of the weight mixing ratio at which the boundary of free DPB begins to appear, was not clear at any *p*H employed in this study.

Discussion

HSA–SDS

Electrophoretic Patterns at *p*H 5.6 and 6.8.—Patterns at these two *p*H values and in the region where HSA/SDS was between 100/0 and 70/30, are discussed referring to results by Pallansch and Briggs[6] and by Yang and Foster.[5] When HSA/SDS was 97.5/2.5 or 95/5, there was a single boundary. The mobility of the component was larger than that of HSA itself and increased gradually with the amount of SDS. The formula of the complex at HSA/SDS = 95/5 was calculated to be AD_{12} (A:HSA and D:SDS). Assumptions were made that all the detergents used were bound to albumin and that the molecular weight of HSA is 70,000 and of SDS is 288. The fact that the mobility of the component increased means that the composition of the complex changed continuously from AD_1 to AD_{12}. In the region of mixing ratio between HSA/SDS = 95/5 and 80/20 there were two boundaries. Mobilities of the two components were constant. This indicates that the complex which coexisted with AD_{12} was AD_n. Here n is 105/2. The number n, which is expected to be equal to one-half the number of basic groups in HSA,[2] cannot be determined accurately by electrophoresis alone. However, the formula calculated from the mixing ratio value HSA/SDS = 80/20, on the same assumptions mentioned above, is AD_{60}. Between the mixing ratio 80/20 and 70/30, there were also two boundaries. Mobilities of the two boundaries were constant, indicating that the composition of the complex which coexisted with AD_n was AD_{2n}. The complex at the mixing ratio = 70/30 is calculated to be AD_{104} using the same assumptions.

When the mixing ratio was between 70/30 and 45/55, there was a single boundary the mobility of which increased continuously with the detergent concentration. From the value of mixing ratio of HSA/SDS = 45/55, the composition of the complex is found to be AD_{6n}. It is seen that the composition of the complex changed continuously from AD_{2n} to AD_{6n}. The complex AD_{4n} suggested in Harrap and Schulman's study[7] by viscosity and light scattering was not found to be a discrete complex in the present electrophoresis.

In the region where the mixing ratio was between 45/55 and 5/95 there were two boundaries. Mobilities of these two components were almost constant, indicating that they are AD_{6n} and SDS. In other words, when $6n$ detergents were bound to one molecule of HSA, no more SDS could be bound. Yang and Foster[5] studied the system BPA–SDBS (sodium dodecylbenzenesulfonate) by equilibrium dialysis. From a table in their paper, it is calculated that 98-88% of the detergent ions used were bound to BPA in the region of mixing ratio (BPA/SDBS) between 100/0 and 93/7 and that 85-81% of the detergent ions used were bound in the region between 92/8 and 36.5/63.5. It was assumed that the molecular weight of BPA is 69,000 and of SDBS is 348. Since, as they stated, the detergent used was not homogeneous and since it is not known whether the affinity of SDBS to BPA is equal to the affinity of SDS to HSA, the two percentage values mentioned above cannot be applied to the present system. However, it is stated that the maximum number of detergents bound to HSA is actually less than $6n$.

Patterns at Different *p*H Values.—Putnam and Neurath[2] observed two components, AD_n and AD_{2n}, at *p*H 6.8 in the region of albumin excess. Yang and Foster,[5] however, observed only one complex which they interpreted as AD_n at *p*H 7.7. There is a disagreement as to the number of discrete complex. At that time it was not made clear whether this difference was caused by the difference in detergent used or by the difference in *p*H employed.

In the present study at *p*H 8.9 there was a single boundary at HSA/SDS = 95/5, and there were two boundaries in the region between HSA/SDS = 95/5 and 70/30. This shows that complex AD_{12} was formed first and that then the complex AD_{2n} was formed without formation of discrete complex AD_n.[17] This result indicates that the disagreement comes from the difference in *p*H. In other words, there is a *p*H boundary between *p*H 6.8 and 7.7, above and below which the number of the discrete complex changes.

At *p*H 10.6 the presence of AD_{12} was not made clear by electrophoresis. That the precipitate was not formed by DBP when there was a great excess of HSA may suggest that a complex similar to AD_{12} exists.

When the area of free SDS is plotted against the weight mixing ratio in the region of detergent excess, a straight line is obtained (Fig. 4a). The straight line AB crosses the axis at HSA/SDS = 42.5/57.5. This value is very close to HSA/SDS = 45/55, which is one of the boundaries of the electrophoretic pattern division. It also was found that the position of the straight line, and

(17) Since the slower moving boundary disappeared at HSA/SDS = 70/30, we are inclined to conclude that the composition of the faster boundary is AD_{2n}, and not AD_n. This is contradictory to the finding by Yang and Foster that AD_n was discrete. Of course, it cannot be known which is the right formula of the complex formed at HSA/SDS = 70/30, AD_n or AD_{2n}, unless the number of SDS ions bound to HSA is determined accurately by equilibrium dialysis. However, we had two boundaries at *p*H 7.7 and at HSA/SDS = 80/20. The pattern was identical regardless of the ionic strength (0.1 and 0.2) and the temperature (25 and 6°). Patterns obtained were the same as that at *p*H 8.9 and at HSA/SDS = 80/20, but not the same as that at *p*H 6.8 and at the same weight mixing ratio.

accordingly that of crossing point B, was not changed by *p*H insofar as this study is concerned. Since the association constant of the interaction between HSA and SDS was not measured and, further, since the boundary mixing ratio value where free SDS begins to appear was difficult to determine accurately, a quantitative discussion cannot be made. It is interesting to note that HSA was saturated with SDS in the *p*H region between 3.5 and 10.6, however, when the same number ($6n$) of SDS ions was bound to HSA. It is emphasized that HSA was saturated with a constant amount of SDS ion in spite of the fact that the mechanism of interaction in the region of albumin excess changed at several *p*H values.

Precipitation Curve.—In Fig. 5 it is seen that the precipitation curves at *p*H 3.5 and *p*H 4.2 are superimposed when albumin is in excess and that the precipitate begins to appear at a common mixing ratio value, *viz.*, at point A in the figure. Since the mixing ratio value at this point is HSA/SDS = 95/5, the composition is AD_{12}. This mixing ratio agrees with the first boundary value of the electrophoretic pattern division at *p*H 6.8. The mobility of the complex formed in the region of mixing ratio between 100/0 and 95/5 decreased with increase of the amount of detergent, indicating that the composition of the component changed continuously. In other words, the complex, having a composition between AD_1 and AD_{12}, is not precipitated even in the acid side of the isoelectric point. Hence the complex AD_m ($m \leqq 12$) exists on both the acid and alkaline sides of the isoelectric point.

HSA–DPB

Since the electrophoretic pattern at *p*H 6.8 was not highly enantiographic, only a qualitative discussion can be given below. When HSA/DPB was between 100/0 and 93/7, the mobility of the component decreased with increase of the amount of detergent, finally attaining a constant value. This suggests that a complex similar to those between AD_1 and AD_{12} in the system HSA–SDS was formed. Since it was observed that the relative area of the boundary changed with the mixing ratio, it can be stated at least that the interaction is stepwise in this system also.

The same interaction was observed at *p*H 8.0. In the system HSA–anionic detergent, the mechanism of the interaction differed at *p*H 6.8 and 7.7, but there was no appreciable difference at *p*H 6.8 and *p*H 8.0 when the cationic detergent was used. The most interesting result was obtained at *p*H 4.4. It is seen in Fig. 4b that the percentage of N plus F_1 decreased when the amount of DPB was increased. Here the mobilities of N and F_1 were constant, and the ratio of areas of N to F_1 was also constant. Thus it is concluded that the cationic detergent converted N into F form.[18]

When HSA or egg albumin was mixed with SDS, precipitate appeared instantly on only the acid side of the isoelectric point. Hence, when the protein and the cationic detergent were mixed, it was expected that the precipitate would appear only on the alkaline side of the isoelectric point, and this was true in the system egg albumin–DPB.[19] However, the expectation was not correct for the present system. When HSA and DPB were mixed on the alkaline side, the amount of precipitate depended on both *p*H and ionic strength.

Conclusions

That the internal structure of serum albumin changes at several *p*H values is realized. It was demonstrated electrophoretically[15] that BPA undergoes an isomerization reaction between *p*H 3.5 and 4.5. There are two or three boundaries (N and F forms) depending upon the ionic strength. The percentage of both forms is a function of *p*H and these two forms are in equilibrium: $F \rightleftarrows N + 3H^+$. It was foundth at HSA undergoes the same isomerization reaction.[19] Below *p*H 3.5 BPA expands.[15] The concept of expansion was first introduced by Tanford[20,21] and by Scatchard[22] based on analysis of the titration curve. This was later verified by various techniques, *i.e.*, viscosity measurements,[23] ultracentrifuge[24] and electrophoresis.[15] Jirgensons[25] stated that a subtle change in internal configuration was revealed at *p*H 7.5–10.5 by optical activity measurements. This change is thought to be reversible denaturation. When the *p*H is above 10, some abnormality occurs in serum albumin. This was pointed out by Tanford, *et al.*,[21] in their study on the titration curve. They interpreted the abnormal behavior as an expansion. Measuring the optical activity of serum albumin as a function of *p*H, Jirgensons and his co-workers[26] showed in a series of papers that the value of optical rotation decreased sharply when the *p*H was above 10. The author also observed when the *p*H was above 10 that the electrophoretic pattern of BPA changed and that the value of sedimentation coefficient decreased.[27] At *p*H 11.4 or higher, irreversible denaturation occurred at room temperature.[25]

Here we have pointed out that the mode of interactions of HSA with anionic and cationic detergents changed discontinuously at several *p*H values. It is considered that the results obtained reflect changes in the internal configuration of serum albumin, changes associated with *p*H as just described. In former section, it was stated that there is a *p*H boundary between 6.8 and 7.7 at which the mechanism of the interaction changes. It is thought that a change in the internal structure of HSA occurs at a *p*H between 6.8 and 7.7. This con-

(18) Exactly speaking, N and F_1 forms were converted into F_2 form. As a first approximation, F_1 was neglected since the percentage of F_1 was very small. The mobility of F_1 was difficult to measure in patterns obtained at HSA/DPB = 90/10 and 85/15.

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clusion agrees with Jirgensons', *viz.*, serum albumin undergoes a subtle configurational change when the *p*H is above 7.5. It is predicted that there will be an interaction with a common mechanism in the *p*H region between 4.5 and this boundary *p*H (*ca.* 7.5) and that there will be an interaction with another common mechanism between *p*H *ca.* 7.5 and *ca.* 10.

At *p*H 4.4 where the isomerization occurs, DPB converted N into F form. Electrophoretic studies were made on the interaction of BPA with SDS between *p*H 3.5 and 4.5 in the region of mixing ratio between 100/0 and 95/5 where no precipitate was formed. It was found that SDS converted the F into N form,[27] and thus the following equation is given for the *p*H region between 3.5 and 4.5

$$N \underset{SDS}{\overset{DPB}{\rightleftharpoons}} F.$$

It was found that anionic and cationic detergents had an opposite effect toward the isomerization reaction—an interesting point. However, much more cationic detergents than anionic detergents were needed to this transformation. This is because the affinity of DPB to HSA is lower than the affinity of SDS to HSA.

At *p*H 3.3, the electrophoretic pattern of the system BPA and a cationic detergent had a single boundary, and that of the system egg albumin and this detergent had two boundaries.[11] (This was confirmed also in the present systems HSA–DPB and egg albumin–DPB.) Foster and Yang interpreted the difference in electrophoretic pattern to be caused by the fact that BPA expands at this *p*H, but egg albumin does not. The same result was obtained at *p*H 10.6. When a mixture of HSA and SDS and a mixture of egg albumin and SDS, with the weight mixing ratio (protein/SDS) of 90/10, were analyzed electrophoretically at this *p*H, the former had a single boundary and the latter two boundaries, *i.e.*, egg albumin and a complex. It was found that egg albumin does not expand at this *p*H.[28] Hence the fact that some abnormality (expansion or degradation) occurs in serum albumin above *p*H 10 is considered to be the reason that electrophoretic patterns of the system HSA–SDS at *p*H 10.6 are quite different from those at *p*H 6.8.

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(28) The sedimentation coefficient of egg albumin at *p*H 10.6 was found to be almost the same as that at *p*H 6.8. This indicates that egg albumin exists in the identical state at these two *p*H values.

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[CONTRIBUTION FROM THE LABORATORY OF THE CHILDREN'S CANCER RESEARCH FOUNDATION]

Compositional Effects on the Configuration of Water-soluble Polypeptide Copolymers of L-Glutamic Acid and L-Lysine[1]

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The synthesis of four high molecular weight random polypeptide copolymers of L-lysine and L-glutamic acid in various mole ratios is described. Studies of the physical-chemical properties of these water-soluble ionic polypeptides indicate that they exist in molecular configurations which depend on their composition and their environment. Infrared spectra of solid films show only a single α-amide frequency when cast from *p*H 3 solutions; the films cast from *p*H 11 solutions have spectra which show the presence of both α- and β-configurations. Infrared spectroscopic studies on D_2O solutions of these polypeptides indicate that the proportion of random configuration compared to α configuration changes as the *p*D is changed from 3 to 10 but no β-configuration is observed. At low *p*H's in solution the proportion in the helical configuration, as measured by optical rotatory dispersion, decreases with increasing L-lysine content. The solubilities, changes in infrared spectra and changes in optical rotation resemble those observed with certain proteins.

In previous communications we have described the synthesis of high molecular weight poly-L-glutamic acid[3,4] and its configurational changes with *p*H,[3,5] heat[5] and water[6] in both the solid state and in solution. We also have investigated configurational changes in a cationic synthetic polypeptide, poly-L-lysine hydrochloride.[7] Because proteins contain both anionic and cationic groups it was interesting to extend these configurational studies of synthetic poly-α-amino acids to species carrying both negative and positive charges in the side chains of the same molecule. Therefore a series of copolymers of L-glutamic acid (L-glu) and L-lysine (L-lys) were synthesized and in this communication we report both the synthesis and the initial configurational studies on these ionic water-soluble polypeptides. An electrophoretic study of L-lys:L-aspartic acid copolymers has been described by Shavit[8] and some properties of one L-lys:L-glu copolymer are being reported by Doty, Imahori and Klemperer.[9]

Infrared spectroscopic studies indicate that these high molecular weight L-glu:L-lys copolymers exist

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