*On the Aggregation of Calf Thymus Histone**

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(Received April 30, 1957)

In recent years, increasing attention has been directed toward protein interactions[1]. Indeed, it has been recognized that many reactions of biological importance, such as enzyme reactions, antigen-antibody reactions, etc., involve interaction of proteins with other proteins or other molecules. By studying the nature of these interactions, not only is it possible to interpret biological phenomena on a molecular basis, but it is also possible to obtain an information about protein structure in an aqueous environment.

It has been found that some proteins associate (aggregate) or dissociate with suitable change in their environment[2]. As these interactions which occur between protein molecules of the same kind are the most simple cases of protein-protein interactions, it would be of value to examine their nature from the physico-chemical point of view.

In the course of studies on purification and characterization of calf thymus histone, it was found that its main fraction, histone I, had a great tendency to aggregate[3]. The protein, when purified cautiously, showed a single homogeneous boundary in an ultracentrifuge at pH values below 7[4,5], but a heavier component appeared when the solution was kept at room temperature, and its amount varied markedly with change in the conditions of pretreatment and measurements. The present author was interested in this finding and studied the aggregation reaction systematically, the results of which are reported in this paper.

Experimental

Material.—The sample used in this study was histone I, i. e., the main fraction of calf thymus histone. The method used for its preparation has already been described[4].

Methods.—Sedimentation and diffusion studies were carried out in the same apparatus described in the preceding paper[5]. Sedimentation measurements were made at room temperature (15—30°C), while those of diffusion were made at 20.0°C. The observed sedimentation and diffusion coefficients were always corrected for the values in water at 20.0°C.

* A part of this work was read before the Symposium on Protein Structure held in Tokyo on November 5, 1955.

1) I. M. Klotz, in H. Neurath and K. Bailey, "The Proteins," New York, Vol. I, p. 727 (1953).

2) D. F. Waugh, *Advances in Protein Chem.*, **9**, 325 (1954).

3) N. Ui, *Biochim. Biophys. Acta.*, **22**, 205 (1956).

4) N. Ui, ibid. in press.

5) N. Ui, This Bulletin, **30**, 801 (1957).

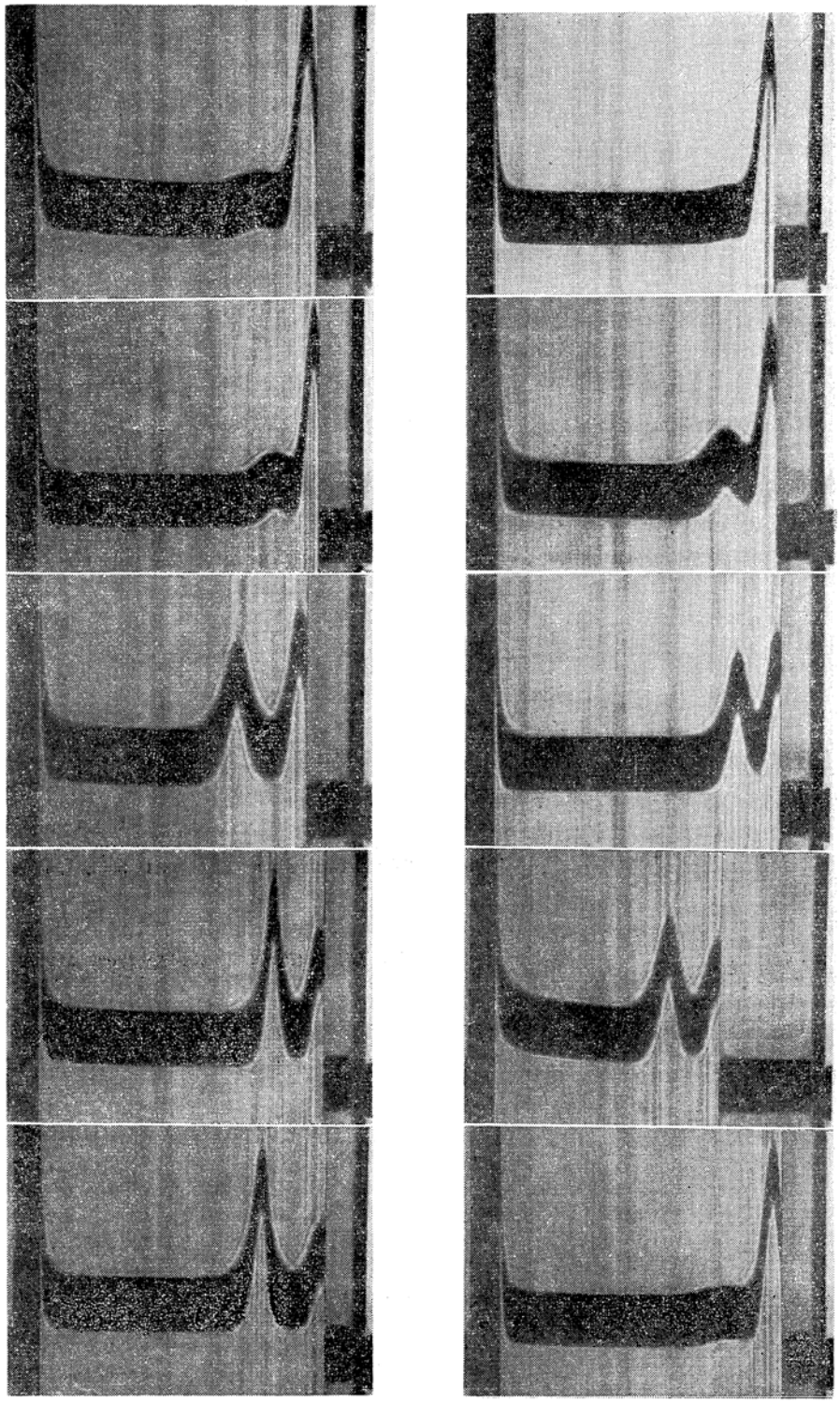

Fig. 1. Sedimentation patterns of aged solutions of histone I at pH 5.0 and ionic strength of 0.1.
Left : Solutions were kept at 30°C in the presence of sodium sulfate (2/300 M) for periods of 30 min., 3, 24, 48 and 168 hr. from top to bottom.
Right : Upper 4 patterns were obtained after aging at 30°C in the absence of sulfate ions for 30 min., 28, 76 and 168 hr. Extreme bottom pattern was obtained after 7 days' keeping at 0°C in the presence of sulfate ions.
Sedimentation proceeds from right to left.

Electrophoretic measurements were made in a Tiselius-type apparatus[6] equipped with a Philpot-Svensson's schlieren optical system[7], and a medium cell with a tall center section[8] was used.

Viscosity was measured at 30.0°C in an Ostwald viscosimeter.

Results

A. Aggregation at 30°C at pH 5.0 and Ionic Strength 0.1.—When a 1 per cent. solution of histone I in an acetate buffer of pH 5.0 at ionic strength of 0.1 was allowed to stand at 30°C and examined in an ultracentrifuge, a faster component with a sedimentation coefficient of 8—10 S was observed besides the original component of this preparation (about 2 S) as is shown in Fig. 1. The amount of the faster component increased slowly with time of aging at 30°C, and reached 55 per cent. of the total protein (see Fig. 2, curve II).

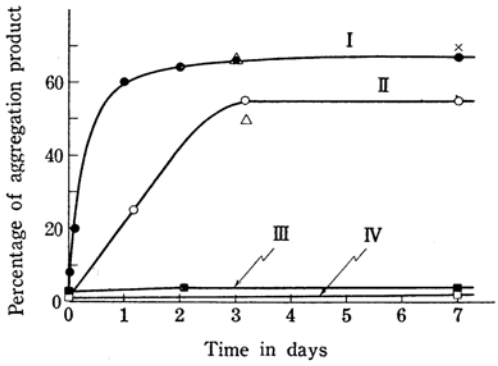

Fig. 2. Increase of the aggregation product with time at pH 5.0 and ionic strength of 0.1.
Curve I : 30°C, in the presence of sulfate ions.
Curve II : 30°C, in the absence of sulfate ions.
Curve III : 0°C, in the presence of sulfate ions.
Curve IV : 0°C, in the absence of sulfate ions.

Sulfate ion was found to be an effective aggregating agent; if an acetate buffer of the same pH and total ionic strength as before but containing sodium sulfate (2/300 M) was used as a solvent, the faster component appeared more rapidly and the amount reached 67 per cent. after one or two days (see Fig. 2, curve I). Some of the sedimentation patterns are shown in Fig. 1.

In contrast with the observation at 30°C, the aggregation did not proceed appreciably at 0°C (see curves III and IV in Fig. 2). Although a small amount (2—3 per cent.) of aggregation was observed in this experiment, it would be due to the high temperature (nearly 30°C) at which the sedimentation was performed. In fact, no faster boundary was found when another solution of histone I was kept for 25 days at 0°C and examined at a lower temperature.

In Fig. 3 is shown the sedimentation coefficient of the faster component as a function of its amount. It was found that the more the aggregation proceeded, the higher was the sedimentation coefficient obtained.

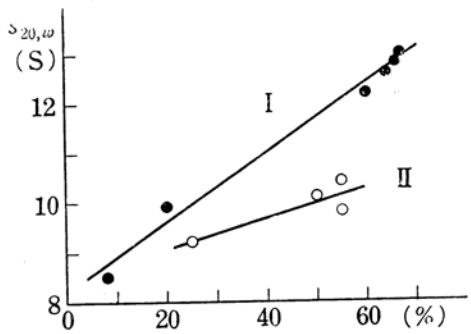

Fig. 3. Relation of sedimentation coefficient of the aggregate with its amount.
Curve I : Aged at 30°C in acetate-Na_2SO_4 buffer of pH 5.0 at ionic strength of 0.1.
Curve II : Aged at 30°C in acetate buffer (sulfate-ion free) of pH 5.0 at ionic strength of 0.1.

The aggregation was not reversed by lowering the temperature. Therefore, when solutions of aggregated histone were examined before and after keeping them at 0°C for 4 days, the amount of aggregate was found to be nearly the same (see marks △ in Fig. 2)*. It was also found that the aggregate, which had been produced in the solution containing sodium sulfate, did not dissociate when sulfate ions were removed by addition of barium chloride (see mark × in Fig. 2).

B. Effect of Variation in Temperature.—Fig. 4 shows the amounts of aggregate formed when a 1 per cent. solution

6) A. Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).
7) J. St. L. Philpot, *Nature*, **141**, 283 (1938); H. Svensson, *Kolloid-Z.*, **87**, 181 (1939).
8) L. G. Longsworth, *Chem. Revs.*, **30**, 323 (1942).

* In Fig. 2, the amounts of aggregate are plotted against the time kept at 30°C. As the aggregation was not reversed by cooling, it was not necessary to perform sedimentation experiments at the same temperature as in the case of the aging. A lower temperature was rather desirable to prevent progress of aggregation during the measurements.

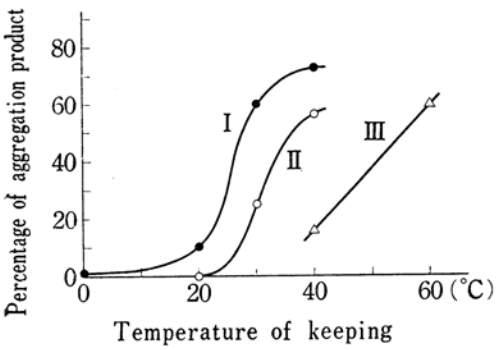

Fig. 4. Effect of temperature upon aggregation.
Curves I and II: Aged for 24 hrs. at pH 5.0 and ionic strength of 0.1, in the presence and absence of sulfate ions.
Curve III: Aged for an hour at pH 5.0 and ionic strength of 0.1, in the absence of sulfate ions.

of histone I in acetate buffers (pH=5.0, ionic strength=0.1) with and without sodium sulfate (2/300 M) were kept at various temperatures. Curves I and II were obtained by keeping the solutions for 24 hours, while curve III was obtained after one hour's keeping.

It was clear that the higher the temperature, the higher the extent of aggregation. At 20°C, histone I did not aggregate during 24 hour's keeping in the absence of sulfate ion, but the aggregation occurred by aging for a longer time (8 per cent. after 4 days' keeping). A lower temperature than 20°C was necessary to keep the solution free from the aggregate for long time.

At higher temperatures, it was found that, not only was the extent of aggregation increased, but also the reaction rate was accelerated. For instance, the initial slope of the amount of aggregate vs. aging time curve was steeper at 40°C than at 30°C. At 40°C, the amounts of aggregate reached 82 per cent. in the presence of sulfate ions and 62 per cent. in its absence, which were higher than those obtained at 30°C.

C. Effect of Variation in Protein Concentration.—A series of histone I solutions in the same solvents as before at various protein concentrations were examined after keeping at 30°C for 24 hours.

As shown in Fig. 5, a higher concentration favored the aggregation. In the presence of sulfate ions, the extent of aggregation in 2 per cent. solution was lower than that in 1 per cent. solution, but it might be due to the decrease in

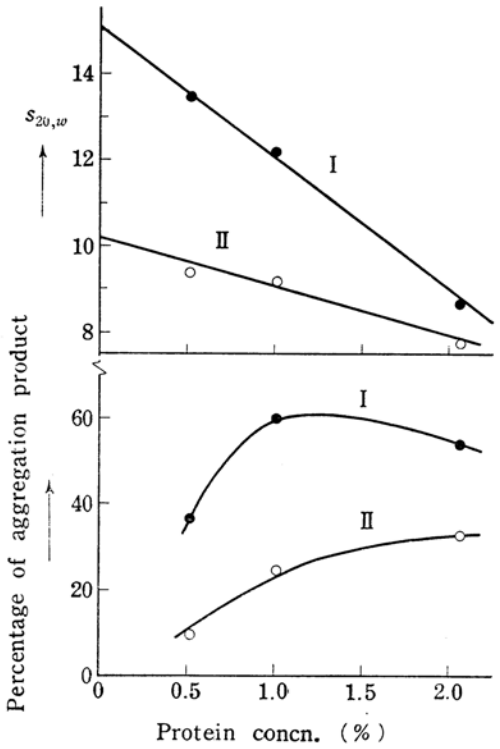

Fig. 5. Effect of protein concentration upon aggregation.
Aged at 30°C for 24 hrs. at pH 5.0 and ionic strength of 0.1, in the presence (curve I) or absence (curve II) of sulfate ions.

the molar ratio of sulfate to the protein with increase in protein cencentration, since the concentration of sodium sulfate was always kept constant (2/300 M) in this experiment.

D. Effect of Variation in Ionic Strength of the Solution.—Effect of ionic strength was studied at pH 5.0 with 1 per cent. solutions of histone I. The concentration of sodium sulfate, if present, was kept always constant at 2/300 M.

The amounts of aggregate produced during 24 hours' aging at 30°C are plotted against ionic strength of the solution in Fig. 6. It was shown that high ionic strength favored aggregation. In the absence of sulfate ions aggregation hardly occurred below ionic strength of 0.05. It was also indicated that the effect of sulfate ion on aggregation was accentuated at lower ionic strengths.

The temperature dependency of this aggregation was again noted; at 0°C the aggregate was not produced appreciably even if the ionic strength was higher and sulfate ions were present (see Fig. 6, curve III).

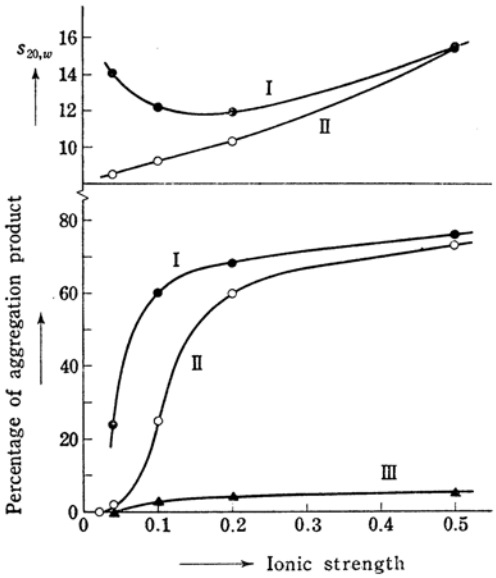

Fig. 6. Effect of ionic strength upon aggregation.
Aged at 30°C for 24 hr. at pH 5.0 in the presence (curve I) or absence (curve II) of sulfate ions. Curve III is that of aged solutions at 0°C.

The sedimentation coefficient of the aggregate also depended upon the ionic strength of the solution (see upper figure in Fig. 6), and a higher value was obtained at a higher ionic strength. In the case of solutions containing sulfate ions, however, sedimentation coefficient vs. ionic strength curve showed a minimum, suggesting the complicated effect of sulfate ion upon aggregation.

E. Effect of Adding Sodium Sulfate.—In view of the finding that the aggregation of histone was greatly accelerated by the presence of sulfate ions, a different amount of sodium sulfate was added to the solution of histone I (pH 5.0) and the effects were compared.

When sodium sulfate was added to a concentration higher than 2/300 M, no appreciable change was observed in the amount of the aggregate formed after 48 hours' aging at 30°C, but the sedimentation coefficient of the aggregate was definitely higher at higher concentration of sulfate ions; for instance, 17.0 S was obtained for 5/300 M sodium sulfate instead of 11.8 S for 2/300 M (total ionic strength was equal to 0.1).

Even a slight addition of sulfate ions was effective for the aggregation. Therefore, histone I sulfate aggregates more easily than its chloride. However, it might be noted that the aggregation of histone I chloride in the acetate buffer (without sodium sulfate) was not due to a slight contamination of sulfate ions in the solution; a curve similar to curve II in Fig. 2 was obtained when the acetate buffer containing barium chloride was used and aged at 30°C.

F. Effect of Variation in pH.—Dependence of the aggregation of histone I upon pH was studied by using buffer solutions of constant ionic strength of 0.1*. Protein concentration was always kept at 1 per cent. and the concentration of sodium sulfate, if present, was 2/300 M.

The amount of the aggregate formed after

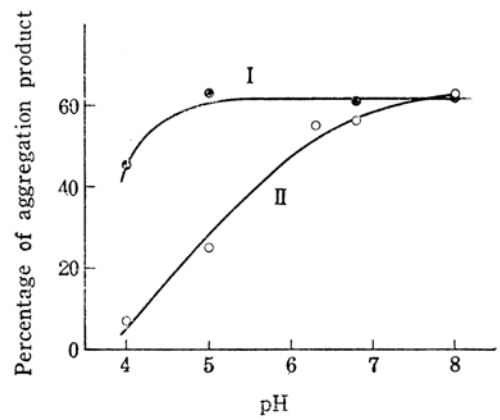

Fig. 7. Effect of pH upon aggregation.
Aged at 30°C for 24 hr. at ionic strength of 0.1 in the presence (curve I) or absence (curve II) of sulfate ions.

24 hours' aging at 30°C is plotted in Fig. 7 as a function of pH. It was noted that higher pH favored aggregation in absence of sulfate ions, but the amount of aggregate found was almost independent of pH values when sodium sulfate was added.

It was also found that some electrolytes caused marked influence upon the aggregation; a gel-like precipitate formed after keeping histone I at 30°C in a phosphate buffer of pH 7.7 or in a veronal buffer of pH 9.0.

As described in the preceding paper[6], histone I showed the presence of a faster component when sedimentation experiment was performed in an alkaline solution above pH 7 except in tris(hydroxymethyl)aminomethane buffer, even if the solutions had not been exposed to a higher temperature. In some cases, especially when pH was 9—10, a precipitate was

* The Following buffers were used in this study: pH 4.0 and pH 5.0, acetate buffer; pH 6.3, NaCl solution (unbuffered); pH 6.8, cacodylate buffer; pH 8.0, tris-(hydroxymethyl)aminomethane buffer.

formed by several days' keeping at 0°C. It was also found that the aggregate disappeared when the solution was soon brought to pH 5.0, so the reversibility of aggregation was suggested.

G. Reversibility of the Aggregation Reaction.—As stated before (see section **A**), the aggrgation was not reversed by lowering the temperature. However, a disaggregation occurred when an aggregated solution was brought to a lower ionic strength by dialysis. Results are shown in Table I.

TABLE I
DISAGGREGATION OF AGGREGATE BY DIALYSIS
pH : 5.0 (acetate buffer) ;
Protein concn. : ca. 1%

Aging			Amount of aggregate after dialysis against buffer with ionic strength of		
Ionic strength	Time at 30°C	Aggregate formed	0.02	0.04	0.1
1.0	72 hr.	73%	—	—	30%
0.1	72	50	—	24%	—
0.1	96	55	15%	—	(15%)*
0.1	24	20	—	—	20%**

* This value was obtained on the solution which had been re-adjusted to ionic strength of 0.1 after being brought to ionic strength of 0.02 by dialysis.

** The solution was once brought to pH 2 by addition of hydrochloric acid and then dialyzed against the acetate buffer of ionic strength 0.1.

H. Molecular-kinetic Properties of the Aggregates.—In order to obtain some information on the size and the shape of the aggregates, sedimentation and diffusion experiments were made with 1 per cent. solution of histone I in an acetate buffer of pH 5.0 at ionic strength of 0.2. The solution had been kept for 4 days at 30°C in the presence and the absence of sodium sulfate (2/300 M). The amount of aggregate formed did not decrease with dilution.

In Fig. 8 is plotted the sedimentation coefficient of the aggregate against concentration, and the sedimentation constant at infinite dilution was calculated by extrapolation.

Diffusion curves markedly deviated from Gaussian curves, as the solution also contained the unaggregated protein. However, since the diffusion coefficient of the unaggregated histone I is known[5], the diffusion coefficients of the aggregates could be calculated, although not accurately, making use of the weight-averaged diffusion coefficients (obtained by the second-moment method) as well as the composition of the solutions determined by sedimentation analysis. The results are shown in Table II. As the diffusion patterns did not show concentration dependency of the diffusion coefficients of the constituents, the values were taken as diffusion constants.

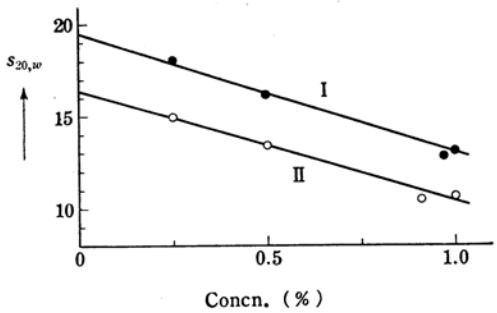

Fig. 8. Concentration dependency of sedimentation coefficient of aggregate at pH 5.0 and ionic strength of 0.2.
Curve I : The aggregate was prepared by aging at 30°C for 4 days in the presence of sodium sulfate (2/300 M).
Curve II : The aggregate was prepared in the same way but in a sulfate ion-free buffer.

TABLE II
DIFFUSION DATA ON AGED SOLUTION OF HISTONE I

Buffer	Aggregate formed (%)	Weight-averaged diffusion coefficient ($D_{20,w}\times10^7$ cm²/sec.)	Diffusion coefficient of aggregate ($D_{20,w}\times10^7$ cm²/sec.)
Acetate	66	1.3_3	1.4_7
Acetate-Na_2SO_4	83	1.6_1	1.7_5

The molecular weight of the aggregate formed either in the presence or the absence of sulfate ions was thus calculated by combining the values of sedimentation and diffusion constants and by assuming that the partial specific volume of the aggregate was the same as unaggregated histone I. In both cases, a value of 1.1×10^6 was obtained (see Table III) and it was found that the aggregate formed in these solutions consisted of about 30 molecules of histone I.

The frictional ratio of the aggregate nearly agreed with that of unaggregated histone. A similar result was obtained when the change in viscosity with time was measured at 30°C. In the absence of sulfate ions (pH=5.0, ionic strength=0.1), η_r

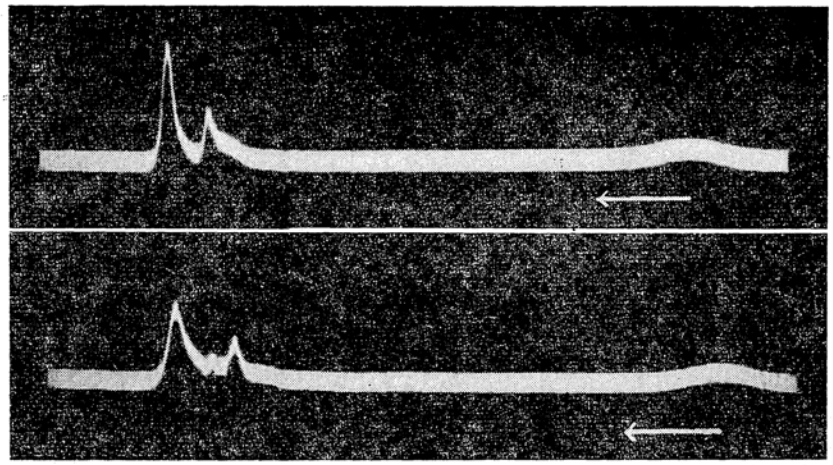

Fig. 9. Electrophoretic patterns of unaggregated (A) and aggregated (B) solutions of histone I in the acetate buffer of pH 5.0 at ionic strength of 0.1. Only ascending patterns are shown.

TABLE III
MOLECULAR-KINETIC DATA ON AGGREGATED HISTONE I

Buffer	$s_{20,w}$ (S)	$D_{20,w} \times 10^7$ (cm²/sec.)	M	f/f_0
Acetate	16.4	1.3_3	1,150,000	2.30
Acetate-Na_2SO_4	19.7	1.6_1	1,140,000	1.91

(viscosity ratio) of 1.18 per cent. solution increased slightly, i. e., from 1.20 to 1.35 after 120 hours. Similarly, when sulfate ions were present, η_r of 1.16 per cent. solution changed from 1.18 to 1.25 after 32 hours' keeping.

I. Electrophoretic Behavior of the Aggregate.—When the aggregation of histone I took place, some change in electrophoretic patterns were observed. An example is shown in Fig. 9.

Although even unaggregated histone I did not show a single boundary (see Fig. 9 A), the electrophoretic patterns became more complex when aggregation occurred (Fig. 9 B); the patterns shown in Fig. 9 were obtained in the acetate buffer of pH 5.0 at ionic strength of 0.1 using the solution which had not been exposed to a high temperature and the solution which had been kept at 30°C for 2 days (the latter solution contained the aggregate about 24 per cent.). It was found that the fastest boundary in the case of the aggregated solution moved to the cathode faster than the fastest component of unaggregated histone I*. A similar variation in electrophoretic pattern with aggregation was observed at other ionic strengths, at a different temperature (25°C), or in the presence of sulfate ions. Therefore, it was concluded that the aggregate had an electrophoretic mobility higher than unaggregated histone at pH 5.0.

Discussion

It is indicated by the results of ultracentrifugal studies described above that histone I produces a rapidly sedimenting component with aging at a temperature higher than about 20°C at the expense of the original component of this protein (2 S), provided that the ionic strength of the solution is not too low. As the molecular-kinetic studies had shown that the size of the faster component was definitely larger than the original protein, the reaction occurring would be due to an aggregation.

The sedimentation coefficient of the newly formed component increased continuously as the reaction proceeded and varied with aging conditions. In view of this finding, the term "aggregation" was used throughout this paper in preference to "association" (for the definition of "aggregation" and "association", see the reference[2]). However, the fairly regular nature of the reaction was suggested, as the aggregate always showed a single boundary with moderate sharpness in an ultracentrifuge; its sedimentation coefficient and the amount were constant so far as the aging conditions were the same.

The aggregation proceeded at a rate sufficiently small to permit the estimation

* The electrophoretic mobility (descending) of the fastest component was calculated as 8.0×10^{-5} cm²/sec. volt in the absence of the aggregate, while 8.6×10^{-5} in the case of the aggregated solution.

of the degree of aggregation at desired levels by sedimentation analysis, and this slowness was one of the main features of this reaction. High temperature or high ionic strength was highly effective in promoting the aggregation and an equilibrium was thereby rapidly established. However, the aggregation could not be reversed by the lowering of the temperature. Disaggregation only occurred when the electrolyte concentration of the solution was reduced by dialysis*. Therefore, it seems that the process involves an equilibrium dependent on ionic strength between the monomer and the aggregate. As the rate of aggregation was slow, it might also be suggested that some modification of the protein precedes aggregation. The experimental results presented here would be accounted for if this conversion is favored by high temperature, and no significant change in the size and the shape of the molecule is assumed to occur by this process. However, as a temperature-dependent modification of calf thymus histone has not been proved, further study is needed to check this conclusion.

Of interest are the forces involved in forming the aggregate. Histone I has an isoelectric point definitely higher than pH 10.3 as revealed by electrophoresis[9]. When amino acid composition of this preparation is assumed to be the same as that of "arginine-rich histone" of Daly and Mirsky[10], it follows that histone I has about 78 positively charged and 21 negatively charged groups per molecule at pH 5.0 (net charge=+57). Therefore, strong repulsion between molecules is expected to exist at this pH, and it must be overcome by specific attractive forces in oder that an appropriate interaction may occur. The contribution of van der Waals' forces or hydrogen bonds to the formation of aggregate might be considerable, although electrostatic attraction between oppositely charged groups, which are disposed appropriately, would also be responsible. In this connection, it might be pertinent to describe our finding that the aggregation was prevented by the presence of urea, which is a well-known hydrogen bond breaking agent; when a solution of histone I was kept at 30°C for 4 days in the presence of 5 M urea (pH=5.0, ionic strength 0.1), no faster sedimenting boundary was observed and only a single sedimentation boundary with the normal sedimentation coefficient of about 2 S was found after the aged solution was dialyzed against the acetate buffer of pH 5.0 and ionic strength 0.1.

It is also to be noted that the suitable configuration of the protein molecule would be required in order that the aggregate might be stabilized by forming a number of bondings due to short range attractive forces (such as hydrogen bonds or van der Waals' forces). Since an electrostatic, repulsive force between charged groups is less prominent at a high ionic strength or a high pH, the aggregated form must become favorable when ionic strength or pH is elevated.

The specific effect of sulfate ions on promoting the aggregation should be emphasized. As the electrophoretic mobility of histone I (unaggregated) at pH 5.0 was found to be markedly diminished by the addition of sodium sulfate[9], it is clear that a large number of sulfate ions are bound with one molecule of histone I. Accordingly, the electrostatic repulsive force between molecules would not be strong enough when sulfate ions are present. It might be also probable that sulfate ions in some manner stabilize the modified form of histone I, which was assumed in the above discussion to be an intermediate in the aggregation reaction. Another bivalent anion, dibasic phosphate ion, was also found to be an effective aggregating agent.

The aggregation product was found to consist of fairly a large number of histone I molecules; i.e., the number of units was about 30 when calculated for the preparation aged at 30°C for 4 days in the presence or the absence of sulfate ions (pH=5.0 and total ionic strength=0.2). Although exact shape of the aggregate is not known, the frictional ratio, which depends upon both shape and hydration, was found to be similar to that of the unaggregated histone I. Electrophoretic mobillty at pH 5.0 only slightly increased with the aggregation. Thus, no significant change except size was found to occur with aggregation. However, it was noticed that histone I solution solubilized sparingly water-soluble materials, such as dimethylaminoazobenzene, especially under conditions in which the aggregation is favorable (unpublished

* The aggregate formed at pH's higher than at low temperature disaggregated when the solution was brought to lower pH's. However, the aggregation in an alkaline solution became more or less irreversible after keeping the solution for a longer time.

9) N. Ui, This Bulletin, in press.

10) M. M. Daly and A. E. Mirsky, *J. Gen. Physiol.*, **38**, 405 (1955).

data). A similar phenomenon has often been reported in an aqueous solution of soap or detergent[11], and is thought to be due to the formation of micelles through the mutual association of hydrophobic and hydrophilic groups in which a sparingly water-soluble material is taken in. On the analogy of this interpretation, it might be possible that the molecules of histone I are specifically arranged in the structure of the aggregate to take in these materials. However, as the interpretation is a matter of speculation, more work is necessary as to the structure of the aggregate.

Since the aggregation of histone I proceeds even at room temperature, especially when the ionic strength of the solution is high, the temperature should be kept as low as possible during the preparation of histone. At pH's higher than 7, the aggregation occurs even if the solution is kept cold and becomes more or less irreversible with time. Therefore, the method for the preparation of histone which takes advantage of its precipitability in an alkaline solution is not recommended. A faster sedimenting component of histone with a sedimentation coefficient much higher than 2 S, which has often been reported by other investigators (see D scussions in the preceding paper[5]) seems to be due to the aggregation that occurred during the isolation of the protein or in the solution in which measurement was performed.

In agreement with the present study, the aggregation of histone has also been pointed out by Cruft, Mauritzen and Stedman[12] and by Davison, James, Shooter and Butler[13]. The former workers came to this conclusion by diffusion measurements which were performed at various pH's. Davison et al. took advantages of sedimentation and other methods, but no detailed data have been reported. They also interpreted the complicated electrophoretic patterns, which were obtained with unfractionated histone at various pH values, by assuming a complex formation between different fractions of histone, and suggested that the greater part of the aggregation reaction involves both 2 S and 1 S component. However, as the electrophoretic patterns were highly complicated[9], more extensive work seems necessary to confirm Davison et al's view.

It is of interest to compare the results of the present study with those of similar studies on other proteins. While association or aggregation occurs with several proteins, it has been known that, only in a few cases of these interactions, an equilibrium is attained at such a slow rate as described in this paper. Johnson et al.[14] have shown that an association-dissociation equilibrium exists in the solution of arachin, a seed globulin in peanut, and that the rate of association or dissociation was slow. It was also shown that pH and ionic strength as well as sulfate ion strongly changed the state of equilibrium and reaction rate[15]. As to the aggregation of conalbumin with acid, Cann et al.[16] showed that the ability of positively charged conalbumin molecules to aggregate resulted from structural modification when the protein was exposed to acidic media. The remarkable effect of sulfate ion upon this aggregation was also stressed. Specific effects of some electrolytes upon a more rapidly attained association-dissociation equilibrium have been reported with hemocyanin[17], insulin[18] and salmine[19].

Summary

The aggregation reaction of histone I, i. e., the main fraction of calf thymus histone, was studied by means of sedimentation, diffusion, viscosity measurement and electrophoresis.

The aggregation was found to proceed usually at a temperature higher than 20°C, although it occurred at pH's above 7 even at 0°C. High temperature, pH, ionic strength or protein concentration promoted the reaction. It was also found that sulfate ion was an effective aggregating agent.

The aggregation was not reversed by lowering the temperature, but disaggregation occurred when the ionic strength of the solution containing the aggregate was reduced by dialysis.

The aggregate was rather large in size.

11) H. B Klevens, *Chem. Revs.*, **47**, 1 (1950).
12) H. J. Cruft, C. M. Mauritzen and E. Stedman, *Nature*, **174**, 580 (1954).
13) P. F. Davison, D. W. F. James, K. V. Shooter and J. A. V. Butler, *Biochim. Biophys.* Acta, **15**, 415 (1954).
14) P. Johnson and E. M. Shooter, ibid., **5**, 361 (1950); P. Johnson, E. M. Shooter and E. K. Rideal, ibid., **5**, 376 (1950).
15) P. Johnson and F. J. Joubert, *J. Polymer Sci.*, **7**, 605 (1951).
16) J. R. Cann and R. A. Phelps, *Arch. Biochem. Biophys.*, **52**, 48 (1954); R. A. Phelps and J. R. Cann, ibid., **61**, 51 (1956).
17) S. Brohult, *J. Phys. & Colloid Chem.*, **51**, 206 (1947).
18) E. Fredericq and H. Neurath, *J. Am. Chem. Soc.*, **72**, 2684 (1950).
19) N. Ui, *Repts. Institute Science and Technol., Univ. of Tokyo*, **8**, 255 (1954).

It consisted of about 30 molecules of histone I under certain aging conditions (pH 5.0) and had nearly the same frictional ratio as that of the monomer. Electrophoretic mobility slightly increased with aggregation at pH 5.0.

On the basis of the data presented, a possible mechanism in forming the aggregate was discussed.

The author wishes to express his sincere thanks Professor Itaru Watanabe for his valuable suggestions and encouragement. His thanks are also due to the Research Laboratory of Precision Machinery and Electronics, Tokyo Institute of Technology, for the kind permission to use their Spinco ultracentrifuge. The expense of this research was partly defrayed by a Grant in Aid for Scientific Research from the Ministry of Education, to which the author's thanks are due.

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