Ultracentrifuge Studies of Histone Fractions from Calf Thymus Deoxyribonucleoprotein

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Molecular weights and sedimentation coefficients of four major fractions of calf thymus histones were measured. The minimum molecular weights were determined in concentrated solutions of guanidine hydrochloride. The results indicate that, with the possible exception of fraction F3, the fractions are heterogeneous. Comparisons in 0.1M-sodium chloride suggest that fraction F1 does not aggregate and show that fractions F2(a) and F3 aggregate to form larger complexes than does fraction F2(b). The degree of aggregation of each fraction is independent of pH in the range pH 1–7. Detailed studies with fraction F2(b) have confirmed that the change in sedimentation coefficient observed as the sodium chloride concentration of the solution is increased results from increases in the apparent molecular weight of the sedimenting units. It has been found that the molecules of fraction F2(b) are present as single molecules only in sodium chloride solutions of 33mM or less. At these low concentrations the effects of charge greatly increase the concentration dependence of the sedimentation rate; the results can, however, be interpreted by using the theory developed by Alexandrowicz & Daniel (1963) and Daniel & Alexandrowicz (1963).

During the last few years a considerable amount of effort has been devoted to the improvement of methods of isolation and fractionation of the histones of calf thymus deoxyribonucleoprotein (cf. Hnilica, 1967*a*; Butler, Johns & Phillips, 1968). These low-molecular-weight basic proteins have now been divided into four or five groups of molecules, each group being fairly reproducible from preparation to preparation and different groups being characterized by different compositions, electrophoretic mobilities and chromatographic characteristics. Groups of workers have unfortunately applied differing terminology to describe the fractions. In this paper we have used the nomenclature of Johns (1964*a*) and Phillips & Johns (1965), in which fractions are denoted as F1, F2(b), F2(a) and F3. The relationship between these fractions and those obtained by other workers has been tabulated by Butler *et al.* (1968). The four fractions cannot be regarded as homogeneous since subfractionation can often be achieved (Johns, 1964*b*; Kinkade & Cole, 1966; Phillips & Johns, 1965; Butler, Power & Palau, 1967; Johns, 1968). It is likely that each of the main fractions consists of groups of molecules of similar molecular weights and composition.

Early work on the physical characterization of unfractionated and partially fractionated histones

was limited in value because of the many possibilities of intermolecular aggregation, proteolytic degradation and non-histone contamination (Butler, Davison, James & Shooter, 1954; Ui, 1956, 1957*a*; Cruft, Mauritzen & Stedman, 1958*a,b*). Despite the advances made in methods of fractionation little systematic work has been reported on physico-chemical parameters. Fraction F1, which alone of the main fractions does not appear to form aggregates in solution, has been studied by five groups of workers (Ui, 1956, 1957*a,b,c*; Trautman & Crampton, 1959; Satake, Rasmussen & Luck, 1964; Teller, Kinkade & Cole, 1965; Haydon & Peacocke, 1968), who have reported molecular weights from 8400 to 21000. Haydon & Peacocke (1968) have demonstrated that the variation in the reported molecular weights arises primarily from neglect of the effects of the high positive charge on the histone molecules and that the true molecular weight for fraction F1 in solution is probably near 20000. In his review, Hnilica (1967*b*) reports that the molecular weights of the three fractions F2(b), F2(a) and F3 measured in 5M-guanidine hydrochloride are all about 13000, the error of the measurement being ± 3000 . No details of the degree of heterogeneity are given.

It was decided to determine whether fractions

F2(a), F2(b) and F3 were homogeneous with respect to molecular weight. Examination of such fractions dissolved in 5.2M-guanidine hydrochloride has shown that fractions F2(b) and F2(a) are heterogeneous although the average molecular weights agree approximately with those given by other workers.

Fraction F2(b) was taken as an example of a histone fraction undergoing self-aggregation and the effects of salt concentration on sedimentation equilibrium and sedimentation transport were studied. The effects of the high charge on the molecules in sedimentation-velocity experiments are considered in terms of the theory developed by Alexandrowicz & Daniel (1963) and Daniel & Alexandrowicz (1963).

It may be noted that studies of the interaction of DNA with histone fractions can only be interpreted satisfactorily if the degree of aggregation of the molecules in solution is known.

EXPERIMENTAL

Calf thymus histone fractions F1, F2(a), F2(b) and F3 were prepared by Dr E. W. Johns and Dr D. M. P. Phillips by the method outlined by Johns (1964a). Solutions of the histones at salt concentrations of 0.1M-NaCl or less were prepared by direct dissolution in solvent. For experiments at higher salt concentrations the histones were first dissolved in water, and an equal volume of buffered salt solution at twice the required final concentration was slowly added.

Sedimentation experiments were performed with a Spinco model E analytical ultracentrifuge. Schlieren and interference optics were focused on the mid-plane of the cell. Rotor temperatures were controlled near to or at 20°. Sedimentation-velocity experiments were conducted either at 59780 rev./min. with a Kel F centrepiece, modified for use as a Kegeles-type synthetic-boundary cell (Kegeles, 1952), or at 67770 rev./min. with a double-sector centrepiece of aluminium-filled Epon. Sedimentation coefficients, corrected to standard conditions, 20° in water, $S_{20,w}$, were calculated from the rate of movement of the boundary maximum. Diffusion coefficients were measured in the analytical ultracentrifuge by using a synthetic-boundary cell. Photographs of the boundary were taken at intervals and from these photographs diffusion coefficients were calculated by the height-area method. The diffusion coefficients were corrected to standard conditions, water at 20°, $D_{20,w}$.

Molecular weights of fraction F2(b) dissolved in solutions of different salt concentrations were determined by the method of Archibald (1947) and calculations made by the procedure of Klainer & Kegeles (1955). Molecular weights of the histone fractions dissolved in 5.2M-guanidine hydrochloride were determined by the method of sedimentation equilibrium with short columns (0.3 cm.) of solution (LaBar & Baldwin, 1962). In these latter experiments photographs were taken with interference optics. Equilibrium was obtained within 48 hr. at rotor speeds of 25980 or 31410 rev./min. Partial specific volumes of the histone fractions were calculated from the amino acid composition (Butler, 1964) by the method of Cohn & Edsall (1943) (cf. McMeekin &

Marshall, 1952). The values obtained for the fractions were: F1, 0.757; F2(a), 0.741; F2(b), 0.737; F3, 0.741 ml.g.⁻¹.

RESULTS AND DISCUSSION

Effect of pH on the sedimentation velocity of histone fractions. Sedimentation coefficients of the four main histone fractions, at a protein concentration of 1%, were measured in 0.1M-sodium chloride buffered at various pH values between 1 and 7. Fractions F2(a) and F3 showed considerable heterogeneity at all pH values in the range studied. The main peak of the schlieren diagram was invariably asymmetric and subsidiary, faster-sedimenting, peaks were present at most pH values. The sedimentation coefficients of these subsidiary, faster-sedimenting, boundaries varied in different experiments from 7s to 20s. These high sedimentation coefficients indicate the presence of gross aggregates. In contrast the schlieren diagrams of fractions F1 and F2(b) showed no such pronounced heterogeneity and no subsidiary, faster-moving, boundaries were seen at any pH. The boundary profile of fraction F1 was practically symmetrical, but that of fraction F2(b) was slightly asymmetric, indicating the presence of a small amount of a more slowly sedimenting species. Further work suggested that the slowly sedimenting component accounted for about 5% of the total protein in fraction F2(b).

Fig. 1. Effect of pH on the sedimentation coefficients of calf thymus histone fractions F1 (○), F2(b) (●), F2(a) (△) and F3 (■). Histone fractions were dissolved in 0.10M-NaCl buffered at different pH values with 0.01M-phosphate ($\text{KH}_2\text{PO}_4 + \text{Na}_2\text{HPO}_4$), 0.01M-sodium acetate-HCl or HCl. Histone concentrations were 1% (w/v). Horizontal straight lines are drawn through the average sedimentation coefficients of fractions F1 and F2(b).

Table 1. Recent molecular weights of calf thymus histone fractions reported by various workers

Results from the present work have not been extrapolated to infinite dilution except for the determinations of fraction F2(b) in 6M-guanidine hydrochloride. The concentration, % (w/w), is indicated by figures in parentheses after the quoted molecular weight.

Method	Fraction	...	F1	F2(b)	F2(a)	F3	Reference
Analysis of (amino acid and acetyl)		12 000-14 000		10 000-11 000	9000-12 500	10 000	Phillips (1963)
<i>N</i> -terminal groups							
Sedimentation equilibrium in 0.50M- or 0.25M-NaCl, pH 9.2			22 000				Teller, Kinkade & Cole (1966)
Sedimentation equilibrium in 0.1M- or 0.5M-NaCl, pH 4.5			19 500				Haydon & Peacocke (1968)
Sedimentation equilibrium in 5.2M-guanidine hydrochloride			21 000	12 000	13 000	14 000	Hnilica (1967b)
Sedimentation equilibrium in 5.2M-guanidine hydrochloride			15 500 (0.030) 17 400 (0.071) 17 100 (0.12) 15 300 (0.14)	10 100 (0.11) 12 400 (0.18) 11 600 (0.18)	8300 (0.20)	14 800 (0.18)	This work
Sedimentation equilibrium in 6M-guanidine hydrochloride				8000 (0.27) 11 500 (0.13) 12 600 (0.07)			

Sedimentation coefficients of the main components of the four histone fractions were calculated from the rate of movement of the maximum of the schlieren peak. The results of these calculations are shown in Fig. 1. Regression analysis performed on the data for fractions F1 and F2(b) indicated that there was no significant change of sedimentation coefficient between pH 1 and 7. For fraction F1 $S_{20,w}=0.72s$ (s.d. 0.15) and for F2(b) $S_{20,w}=1.68s$ (s.d. 0.18). Sedimentation coefficients for the main components of fractions F2(a) and F3 were higher than those observed for fraction F2(b), mean values over the range pH 1-7 being 2.1s for fraction F2(a) and 2.4s for fraction F3. The minimum molecular weights of the histone fractions calculated from the analysis of the *N*-terminal groups and acetyl content are very similar, ranging from 9000 to 14000 (Phillips, 1963) (see Table 1). The sedimentation coefficients reported above, however, indicate that the molecular weights of the four fractions in solution differ considerably. It would appear that, with the exception of fraction F1, the histone fractions exist as aggregates in solution in 0.1M-sodium chloride at from pH1 to pH7. The experimental evidence shows that the extent of aggregation is not dependent on pH over the range studied.

Minimum molecular weight of histone fractions. As a preliminary to further work estimates of the molecular weights of the single molecules of the histones were required. To eliminate the gross effects of charge and in an attempt to avoid the formation of aggregates the histone fractions were dissolved in concentrated (5.2 or 6M) guanidine hydrochloride (Tanford, Kawahara & Lapanje, 1967; Castellino & Barker, 1968). After thorough dialysis against this solvent molecular weights were determined at several concentrations of histone by the method of sedimentation equilibrium in short columns (0.3cm.). The apparent weight-average molecular weights calculated for each solution are given in Table 1. The values for fractions F2(b) and F3 are similar to those reported by Hnilica (1967b). Molecular weights found for fractions F1 and F2(a) are less than those previously reported. An indication of heterogeneity was obtained from plots of the logarithm of the fringe displacement ($\log j$) against the square of the radial distance (r^2). An example of a plot of $\log j$ against r^2 for each fraction is given in Fig. 2. Except for fraction F3 each plot exhibits upward curvature, demonstrating that the histone fractions used were not homogeneous. The linear plot observed for fraction F3 suggests that this fraction may be homogeneous. The various molecular weights calculated from these experiments are compared in Table 1. Experiments with fractions F1 and F2(b) were performed at several different protein concentrations in the range 0.03-0.27%

(w/w) to provide information for the determination of the molecular weight at infinite dilution. The scatter of the results obtained with solutions in 5.2M-guanidine hydrochloride was in general too great to allow accurate extrapolation. Results from experiments with fraction F2(b) dissolved in 6M-guanidine hydrochloride could be extrapolated to zero histone concentration. The corrected weight-average molecular weight obtained on extrapolating $1/M_w$ against $(C_m + C_b)/2$, where C_m and C_b are the concentrations at the meniscus and the cell bottom at equilibrium, was 15500. It is clear, as shown for fraction F1 by Haydon & Peacocke (1968), that the concentration-dependence of the observed molecular weights is considerable. Correction for the

effects of concentration would give weight-average molecular weights for fractions F1, F2(b) and F3 greater than the values obtained from *N*-terminal assays. It would appear either that aggregation of the histone molecules is not entirely eliminated in guanidine hydrochloride or that the fractions contain molecules of a wide range of sizes.

Effect of salt concentration on the sedimentation characteristics of fraction F2(b). To obtain more information on the sedimentation characteristics of histone fractions under a variety of conditions fraction F2(b) was selected for detailed study. It may be noted that fraction F1, alone of the histone fractions, does not aggregate in solution (Davison & Shooter, 1956). With fractions F2(a) and F3 on the other hand it is difficult to avoid the formation of gross aggregates.

The results shown in Fig. 1 indicate considerable scatter ($\pm 10\%$) in the measurement of sedimentation coefficients of fraction F2(b). These deviations may have been due to difficulties in the measurement of the positions of the maximum of a rapidly diffusing peak, variations in charge effect resulting from inadequate dialysis of the solutions, or a time-dependent aggregation of histones after dissolution. Since the histones in solution at pH 7 or less are highly charged molecules efficient dialysis is important. For example, at pH 3.7 in 0.1M-sodium chloride, dialysis of one solution increased the observed sedimentation rate by 13%. Since some histone solutions were stored at 4° before use it was necessary to know whether any changes occurred during this storage time. Two solutions of a preparation of fraction F2(b) were made in 0.1M-sodium chloride, pH 2.8. These solutions were stored at 4° and measurements of the sedimentation rate were carried out at intervals over a period of 21 days. The variation of sedimentation coefficient

Fig. 2. Sedimentation equilibrium with short columns of solution (0.3 cm.) of histone fractions dissolved in 5.2M-guanidinium hydrochloride. The equilibrium distribution of each histone fraction is shown as a plot of $\log j$ against r^2 (where j refers to the fringe displacement measured at a radial distance r): $\circ$, F1; $\bullet$, F2(b); $\triangle$, F2(a); $\blacksquare$, F3. The diagram of $\log j$ against r^2 for fraction F2(b) has been displaced vertically downwards by $\log j = 0.5$. Approximate initial concentrations determined by independent synthetic-boundary experiments were for the fractions: F1, 0.14; F2(b), 0.17; F2(a), 0.21; F3, 0.17% (w/w).

Fig. 3. Sedimentation coefficients of histone fraction F2(b) measured at different times after preparation of two separate solutions. Histone was dissolved in 0.10M-NaCl buffered at pH 2.9. The concentration of both solution 1 ($\bullet$) and solution 2 ($\triangle$) was 1% (w/v).

with time of storage is shown in Fig. 3. Statistical analysis of the results indicated that no significant change in the sedimentation coefficient occurred during storage: solution 1, $S_{20,w} = 1.02$ s (s.d. 0.064); solution 2, $S_{20,w} = 1.03$ s (s.d. 0.032). Errors observed in replicate measurements on the same solution are thus somewhat less than those observed on different solutions (Fig. 1). It would appear that about half the error in the measurement of sedimentation coefficients of different solutions arises from difficulties in the measurement of boundary position. The remaining error may be indicative of some variation in the average degree of aggregation of the molecules in different solutions.

It will be noted that the sedimentation coefficients quoted above for solutions of fraction F2(b) in 0.1M-sodium chloride differ from those illustrated in Fig. 1. It was found that histone fractions that had been kept in the freeze-dried state gradually underwent irreversible aggregation. Further work was performed with fresh preparations only.

Solutions of fraction F2(b) were prepared and dialysed at a range of sodium chloride concentrations. The solutions were buffered between pH 2.9 and 3.9. In sedimentation-velocity experiments

Fig. 4. Effect of NaCl concentration on the sedimentation coefficient of histone fraction F2(b). Histone solutions were prepared at the appropriate salt concentration and then dialysed. Sedimentation coefficients were calculated from duplicate determinations. All experiments were performed at a histone concentration of 1% (w/v). Solution pH was 3.4 ± 0.5 . The NaCl concentration is expressed on a logarithmic scale.

with solutions of fraction F2(b) in 0.01M- and 0.05M-sodium chloride the boundaries showed the asymmetry typical of concentration-dependent sedimentation. At higher salt concentrations, 0.10–1.00M-sodium chloride, the single boundaries observed were nearly symmetrical. In 2.00M-sodium chloride gross aggregation occurred, more than 80% of the solute having a sedimentation coefficient greater than 11s. The effect of salt concentration on this histone fraction is shown in Fig. 4. The sedimentation rate increased rapidly with ionic strength, from 0.42s in 0.01M-sodium chloride to 2.53s in 1.00M-sodium chloride. The results suggest that an aggregation process occurs that is dependent on ionic strength, though at the lower salt concentrations the observed increase in sedimentation coefficient is in part due to the decrease of the primary charge effect as the concentration of sodium chloride is increased. The magnitude of this charge effect is discussed below.

To confirm that histone fraction F2(b) undergoes a progressive aggregation, the molecular weight of the histone was determined in solutions of different ionic strength. Two methods were used. First, the diffusion coefficient was measured and, by utilizing the appropriate value of the sedimentation coefficient, the molecular weight calculated by using the Svedberg equation (Svedberg & Pedersen, 1940). Second, the molecular weight was measured by using the method of Archibald (1947). Results from the latter technique give the weight-average molecular weight of the solute in the initial solution. In the first method the sedimentation coefficients were obtained from measurements of the rate of movement of the maxima of the schlieren peaks; calculations based on these data will give molecular weights that are less than the weight-average molecular weight and may approximate to the mean molecular weight of the major component present in the solutions (Pain, 1965; Creeth & Pain, 1967).

Diffusion coefficients were measured for solutions of fraction F2(b) at five different salt concentrations. Molecular weights calculated from diffusion coefficients and the corresponding sedimentation coefficients are given in Table 2.

In the experiments using the method of Archibald (1947) molecular weights were obtained from measurements at the air-solution meniscus only, the refractive-index gradient at the cell bottom being too steep for accurate measurements to be made. In each experiment the apparent molecular weight calculated from successive photographs decreased with time, indicating that the solutes were not homogeneous. The weight-average molecular weight of the solute in the initial solutions was obtained by plotting the observed molecular weight against the square root of time of sedimentation and extrapolating to zero time (Yphantis, 1959).

Table 2. Sedimentation coefficients, diffusion coefficients and apparent molecular weights of histone fraction F2(b) at different salt concentrations, pH 3.4 ± 0.5

All measurements were made at a single protein concentration (1%, w/v). The sedimentation coefficients are means of pairs of measurements. $M_{S,D}$ is the molecular weight calculated from sedimentation-velocity and diffusion data. M_w is the molecular calculated by using the method of Archibald (1947). Values of M_w in 0.01 M- and 0.05 M-NaCl are the means of duplicate experiments.

Concn. of NaCl (M)	0.01	0.05	0.10	0.50	1.00
$10^{13}S_{20,w}$ (sec.)	0.43	0.65	1.04	2.10	2.53
$10^7D_{20,w}$ (cm. ² sec. ⁻¹)	10.30	5.79	5.63	4.58	3.10
$M_{S,D}$	3900	10500	17600	45900	87300
M_w	7800	11300	31000	60000	110000

Fig. 5. Variation of molecular weight with the square root of the time of sedimentation for histone fraction F2(b) at different salt concentrations. Molecular weights were determined by the method of Archibald (1947) and measurements were made at the air-solution meniscus only. The experiments were performed at a histone concentration of 1% (w/v) with solutions of pH 3.4 ± 0.5 . The weight-average molecular weights of the initial solutions are indicated by the intercept on the vertical axis of the lines, which extrapolate the data to zero time of sedimentation.

Typical plots of the data are shown in Fig. 5. The variation of molecular weight with time is more pronounced the greater the salt concentration. These observations indicate that, as the ionic strength of the solutions increased, the average size of the aggregates present increased and the range of molecular sizes increased as well. Apparent weight-average molecular weights obtained from these experiments are compared with the molecular

weights calculated from the sedimentation-velocity and diffusion data in Table 2. As expected, the weight-average molecular weights were greater than those calculated from the Svedberg equation, the difference becoming more marked as the ionic strength increases. The results of these two sets of experiments confirm that fraction F2(b) undergoes a progressive aggregation as the salt concentration is increased.

At the lowest salt concentration, 0.01 M-sodium chloride, the apparent molecular weight obtained is considerably less than the minimum calculated from the N-terminal amino acid analysis (Table 1). This low value results from neglect of the effects of the high charge on the molecules for which no correction has so far been applied. It can be shown (Williams, Van Holde, Baldwin & Fujita, 1958; Erlander & Senti, 1963a,b) that, in sedimentation equilibrium, charged macromolecules exhibit a deviation from ideal thermodynamic behaviour that gives rise to a large second virial coefficient (cf. Haydon & Peacocke, 1968). This non-ideality is manifested by a considerable dependence of apparent molecular weight on concentration. Theory suggests that the molecular weight obtained after extrapolation to infinite dilution approaches, but is still less than, the molecular weight of the macro-ion (Williams *et al.* 1958). Measurements of the concentration-dependence of the molecular weight in 0.01 M-sodium chloride were made. When the results were extrapolated to infinite dilution of histone a molecular weight of 12200 was obtained. Allowance for charge effects may be made according to the procedure of Williams *et al.* (1958, pp. 731-733) in which the complex expression describing equilibrium distribution of solute species is simplified at zero protein concentration to:

$$M_p(c \rightarrow 0) =$$

$$M_p \left\{ 1 - \frac{Z(1 - \bar{V}_s \rho) M_s}{2(1 - \bar{V}_p \rho) M_p} \right\} \left\{ 1 - \frac{Z M_s \cdot R_s}{2 M_p \cdot R_p} \right\}$$

$M_p(c \rightarrow 0)$ is the experimentally observed molecular

weight extrapolated to infinite dilution, M_s , $\bar{V}_s$ and R_s refer to the salt present and M_p , $\bar{V}_p$ and R_p to the protein. R is the specific refractive-index increment. From the amino acid composition it may be calculated that, for $M=12700$, $Z=25$. By inserting these values the correction term has been calculated:

$$M_p (c \rightarrow 0) = 0.80 M_p$$

Applying this equation gives a corrected molecular weight of 15200 for fraction F2(b) in 0.01M-sodium chloride. If it is assumed that the charge/mass ratio of the histone molecules is unaltered in the presence of 6.0M-guanidine hydrochloride then a comparable correction should be applied to the molecular weight determined from sedimentation-equilibrium experiments in this solvent system. If this correction is applied the molecular weight at zero histone concentration in the presence of 6.0M-guanidine hydrochloride becomes 19000. The agreement between the results from the two methods of measurement is not good. Under both these sets of conditions, where aggregation is expected to be at a minimum, the average molecular weight observed is greater than that obtained from end-group analysis (Table 1). On this basis it would appear that fraction F2(b) is heterogeneous, the number-average molecular weight being about 10000–11000 and the weight-average molecular weight, when allowance for Cl^- ions in the protein preparation is made, being between 14000 and 17500.

Charge effects and sedimentation velocity of fraction F2(b). It should be noted that the correction for the effects of charge in the equilibrium experiments (see above) is independent of the salt concentration. In transport experiments, on the other hand, the magnitude of the electrostatic field generated by the relative movements of macromolecules and solvent ions depends on the concentrations of both species. The magnitude of the charge effect in sedimentation-velocity experiments may be determined by measuring the concentration-dependence of sedimentation rate at different ionic strengths. In experiments with fraction F2(b) sedimentation coefficients were measured at protein concentrations in the range 0.2–1.2% (w/v). Salt concentrations from 0.01M- to 0.50M-sodium chloride were used. The solutions were buffered at pH 3.3 with sodium acetate-hydrochloric acid, the buffer concentration being not greater than one-tenth of the concentration of the sodium chloride.

The variation of sedimentation coefficient with concentration may be written:

$$\frac{1}{S} = \frac{1}{S^0} (1 + Kc)$$

Fig. 6. Concentration-dependence of the sedimentation coefficient of histone fraction F2(b) at different salt concentrations. Sedimentation coefficients were calculated for several different initial concentrations of histone for each salt concentration used. The reciprocal sedimentation coefficient has been plotted against the corresponding histone concentration (c). Straight lines were drawn through the data and extrapolated to zero histone concentration. Concn. of NaCl (M): ▲, 0.50; ○, 0.10; ■, 0.033; ▽, 0.015; ●, 0.010. Salt solutions were buffered with sodium acetate-HCl at pH 3.3; the concentration of buffer did not exceed one-tenth of the nominal concentration of NaCl.

where S^0 is the sedimentation coefficient at infinite dilution, c is the concentration of protein and K is a constant. Plots of the reciprocals of the measured sedimentation coefficients against protein concentration for solutions of different ionic strength are shown in Fig. 6. The intercept, $1/S^0$, is much the same for measurements made in concentrations of sodium chloride between 0.01 and 0.033M but decreases in 0.10M-sodium chloride and is even less in 0.50M-sodium chloride. The determinations of S^0 , although unavoidably of low precision, suggest that aggregation is not a major effect at ionic strengths below 0.033M and that the molecular weights measured in solutions containing either 0.01M-sodium chloride or 6M-guanidine hydrochloride are the minimum values that can be obtained for this preparation.

The slopes of the lines in Fig. 6 increase progressively as the salt concentration is lowered. The slope of the line is a composite term that includes both measures of physical interaction and the effects of charge. Alexandrowicz & Daniel (1963) have shown that, in the presence of excess of salt and when secondary charge effects can be neglected, to a first approximation, the initial equation can be rewritten:

$$\frac{1}{S} = \frac{1}{S^0} + \frac{K_p c}{S^0} + \frac{K_e c}{m_s}$$

Fig. 7. Charge effect for sedimentation-velocity experiments with histone fraction F2(b). Points represent the slopes of the straight lines drawn in Fig. 6 (i.e. the concentration-dependence of reciprocal sedimentation coefficient at a particular salt concentration) plotted against the reciprocal of the corresponding salt concentration. The salt concentration includes the contribution to the ionic strength of the medium from the buffer present.

K_p is obtained formally from the concentration-dependence of the hydrodynamic frictional coefficient of the protein, $f=f^0(1+K_p c)$. In the additional term, which relates to the charge effect, m_s is the concentration of salt present (moles ml.⁻¹) and K_e is a constant primarily dependent on the charge/mass ratio of the protein. The magnitudes of the two terms can be found by plotting the slopes of the lines in Fig. 6, $K_p/S^0 + K_e/m_s$, against the reciprocal of the salt concentration. This plot should be a straight line of slope K_e and intercept K_p/S^0 . As is shown in Fig. 7 the results obtained for the histone fraction fall on a straight line. From the slope of this line it was calculated that $K_e = 0.96 \times 10^{10} \text{ sec.}^{-1} \text{ g.}^{-1} \text{ cm.}^3$. Theoretical analysis by Alexandrowicz & Daniel (1963) has shown that K_e may be calculated from:

$$K_e = \left(\frac{Z}{M} \right)^2 \frac{f^\pm}{2(1 - \bar{V}\rho)}$$

where Z is the effective charge on the macromolecule of molecular weight M , $(1 - \bar{V}\rho)$ is the usual buoyancy term and $f^\pm$ is the mean frictional coefficient of the ions of the supporting electrolyte. For

sodium chloride a value of $f^\pm = 1.56 \times 10^{15} \text{ g. sec.}^{-1} \text{ mole}^{-1}$ can be calculated from the diffusion coefficient (Robinson & Stokes, 1958). A value of M/Z can be obtained from the amino acid composition of the histone fraction. Taking the minimum molecular weight calculated from amino acid composition, $M = 12700$, such a unit at pH 3.3 would carry 25 positively charged groups. By inserting the experimentally obtained value of K_e in the equation it was calculated that, for $M = 12700$, $Z = 22$. This value is close to that expected.

The relative magnitude of the charge effect and the frictional term can be illustrated by considering the contributions to the change in the reciprocal sedimentation coefficient, $1/S - 1/S^0$, when the histone concentration is increased from zero to 1%. The contribution to the total difference, $1/S - 1/S^0$, due to the frictional term is $K_p c/S^0$. The value of this term that can be calculated from the concentration and the intercept of Fig. 6 is a constant independent of the ionic strength of the solution. It may be noted that the frictional term (Table 3) is nearly equal to the increase in $1/S$ observed in the experiments in 0.5M-sodium chloride. The term correcting for charge effect, $K_e c/m_s$, can be calculated for each salt concentration used. As is shown in Table 3 this term is much less than the frictional term for the experiments in 0.5M-sodium chloride, but at lower salt concentrations the contribution of this term becomes increasingly important. At the low ionic strength of 0.013 the charge effect is about 6.1 times as large as the frictional term. The marked dependence of sedimentation rate on histone concentration at low salt concentrations is thus due primarily to the charge on the molecules. It should be observed that even at an ionic strength of 0.10 the charge effect is still about equal to the frictional effect. Clearly salt concentrations in excess of 0.1M are required to suppress the effects of the charge on these molecules.

In the above discussion it has been tacitly assumed that M/Z is a constant independent of the degree of aggregation of the molecules. The assumption receives support from the observation that the plot shown in Fig. 6 exhibits no significant curvature.

Table 3. *Magnitude of the charge effect in sedimentation-velocity experiments with histone fraction F2(b)*

The contribution of the frictional ($K_p c/S^0$) and charge effect ($K_e c/m_s$) terms to the increase in reciprocal sedimentation rate ($1/S - 1/S^0$) when the histone concentration is raised from zero to 0.01 ml. g.⁻¹ are given at various ionic strengths. The relative magnitude of the charge effect is shown in the bottom line.

I	0.013	0.017	0.036	0.10	0.50
$1/S - 1/S^0$	0.89	0.71	0.44	0.22	0.15
$K_p c/S^0$	0.125	0.125	0.125	0.125	0.125
$K_e c/m_s$	0.76	0.55	0.27	0.094	0.019
Charge effect friction	6.1	4.4	2.2	0.75	0.07

The method of plotting the data used in Fig. 6 also suggests that K_p/S^0 is a constant. Studies of the dependence of S on c (Fig. 5) clearly show that at salt concentrations greater than 33mM S^0 changes markedly as the degree of aggregation increases. It would therefore appear that K_p and S^0 increase together at the same rate, i.e. that the basic shape of the molecule is preserved during the aggregation process.

Studies of the interaction of histones and DNA have not so far taken into consideration the state of the histone molecules in solution (Huang, Bonner & Murray, 1964; Johns & Butler, 1964; Boublik, Šponar & Šormová, 1967). It seems likely that the nature of the interaction between a histone fraction and its homologous DNA may be to a large extent determined by the degree of aggregation of the histone molecules. For example, a specific interaction between DNA and single molecules of fraction F2(b) could be detected only in experiments performed in solutions in which the salt concentration is less than 33mM-sodium chloride.

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