exhibit sigmoidal binding curves and are characterized by an infinite value of X^* (or its equivalent) at saturating ligand concentrations strongly suggests that these too may belong to the preexisting equilibrium category. Examples include the binding of threonine to the aspartokinase-homoserine dehydrogenase system (Janin and Cohen, 1969; Wampler et al., 1970) and of ATP or UTP to cytosine triphosphate synthetase (Levitzki and Koshland, 1972). Two final points may be made. First, despite the apparent similarity in experimental behavior of alternative models, mathematical expressions are now available which permit computer-simulation of, for example, binding curves and hence distinction in quantitative terms between possible alternatives. The second and related point is that it is hazardous to ascribe mechanistic significance to the form of a binding curve merely on the grounds that it exhibits apparent positive or negative cooperativity.

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Histone Interactions in Solution and Susceptibility to Denaturation†

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ABSTRACT: Histone interactions in solution may depend upon treatments used for purification. Optical rotatory dispersion and sedimentation-velocity measurements have been made in a reference solvent, before and after exposure to various treatments, to investigate histone susceptibility to irreversible denaturation. Some acid conditions and urea and guanidine solutions may denature. Interaction studies performed on nondenatured histones indicate that the dimer, (H4)(H3), and tetramer, $(H4)_2(H3)_2$, dissociate to monomers at low ionic strength. Sedimentation-velocity experiments suggest a model for the $(H4)_2(H3)_2$ tetramer, with a compact semispherical center and four protruding amino-terminal regions. Fractions H2a and H2b interact to form the mixed dimer in equilibrium with monomers. Fraction H2a self-associates readily to dimers, tetramers, and octamers, while fraction H1 associates only weakly to form dimers.

Histone interactions play an essential role in the maintenance of chromatin structure. The role may be that of packaging the DNA and/or nonspecific mediation of the regulation of transcription or replication. Recent studies have begun to describe the structural features of nucleoprotein. Chromatin subfragments have been visualized by electron microscopy of cross-linked chromatin (Olins and Olins, 1974), and isolated following nuclease digestion (Hewish and Burgoyne, 1973; Sahasrabuddhe and van Holde, 1974; Noll, 1974; and Oosterhof et al., 1975). Kornberg (1974) has suggested that these subfragments are the primary structural unit of chromatin and contain two each of histones H4, H3, H2a, and H2b. Partial cross-linking of histones bound to DNA has indicated the proximity of histones H4, H3, H2a, and H2b (Martinson and McCarthy, 1975; Hyde and Walker, 1975; and van Lente et al., 1975); indeed, Thomas and Kornberg (1975) have identified the octamer containing two each of these four histones. These proximal histones interact both with DNA and with each other. Studies of isolated histones in solution may be used to investigate histone-histone interactions. The cross-linking of DNA-bound histones and the solution studies complement each other in that the former elucidates the chromatin structure, while the latter identifies specific histone interactions contributing to that structure.

Solution studies have demonstrated several pairs of histone interactions. We have previously reported the existence of an H4–H3 complex which reversibly self-associates to form the $(H4)_2(H3)_2$ tetramer (Geoghegan et al., 1974; Roark et al., 1974; Roark, 1976). The tetramer has been observed in the similar solution studies of Kornberg and Thomas (1974) and

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D'Anna and Isenberg (1974a). The latter group has also reported an interaction between H2b and H4 (D'Anna and Isenberg, 1973), while several laboratories have reported an association in solution between H2a and H2b (Kelly, 1973; D'Anna and Isenberg, 1974b; Kornberg and Thomas, 1974; Sperling and Bustin, 1975).

Specific histone interactions should require the maintenance of native protein conformation. We and others have expressed reservations concerning studies utilizing histones prepared with procedures often thought potentially denaturing to proteins (Roark et al., 1974; Kornberg and Thomas, 1974). Procedures used to purify histones commonly involve acid extraction of chromatin with subsequent fractionation by apolar solvents or by denaturants such as guanidine. Van der Westhuyzen and von Holt (1971) have described a "gentle" means of histone preparation and partial fractionation that has led to the discovery of the $(H4)_2(H3)_2$ tetramer by our laboratory and Kornberg and Thomas. Obviously, one should determine if other common fractionation procedures lead to denaturation. D'Anna and Isenberg (1974a) have suggested that although denaturation may occur during preparation, spontaneous renaturation occurs, presumably by histone–histone or histone–DNA interactions. In the present work we examine a series of solvent conditions for their ability to produce an irreversible denaturation of calf thymus whole histone. We have chosen as criteria of the native state the maintenance of secondary structure, as determined by optical rotatory dispersion, and the maintenance of the weight-average sedimentation coefficient, $\bar{s}$. The latter measurements should be sensitive to the degree and type of histone interaction. Since histones have no enzymatic or other well-delineated specific functional role, the criteria of native conformation are less than satisfactory. The percent α-helix and $\bar{s}$ of whole histone in a reference solvent is determined before and after exposure to the treatment solvent of interest. Changes in the percent helix and $\bar{s}$ would indicate an irreversible denaturation. No change in these values would indicate either that the histones are not detectably denatured by the treatment, or that renaturation occurs upon return to the reference solvent. We also report here additional histone interaction studies performed by sedimentation.

Experimental Procedure

Calf thymus histones were purified by modifications of the methods of Van der Westhuyzen and von Holt (1971) as described previously (Roark et al., 1974). Briefly, this involves extraction of purified chromatin with 0.5 M $MgCl_2$ (plus 0.05 M histidine, 0.05 M $NaHSO_3$, pH 6); followed by precipitation of DNA by addition of protamine sulfate and dialysis against 0.05 M NaOAc, 0.05 M $NaHSO_3$, 0.01 M EDTA, pH 5.0. Excess protamine is removed by chromatography at room temperature on Sephadex G-50. Proteolysis was inhibited throughout these procedures by 0.05 M $NaHSO_3$ (Panyim et al., 1968; Bartley and Chalkley, 1970) and 0.01 M EDTA to retard pH changes resulting from the HSO_3^-. When necessary, histone solutions were concentrated either by adsorption on carboxymethylcellulose and elution with the acetate–bisulfite buffer plus 1.5 M NaCl or by osmotic removal of solvent by means of dialysis tubing surrounded by dry Sephadex. Some more recent preparations employ 0.1 mM PMSF[1] in place of $NaHSO_3$ for prevention of proteolysis (Nooden et al., 1973), and six washes of chromatin prior to histone extraction with 0.115 M NaCl, 0.01 M histidine, 0.1 mM PMSF, pH 6. Whole

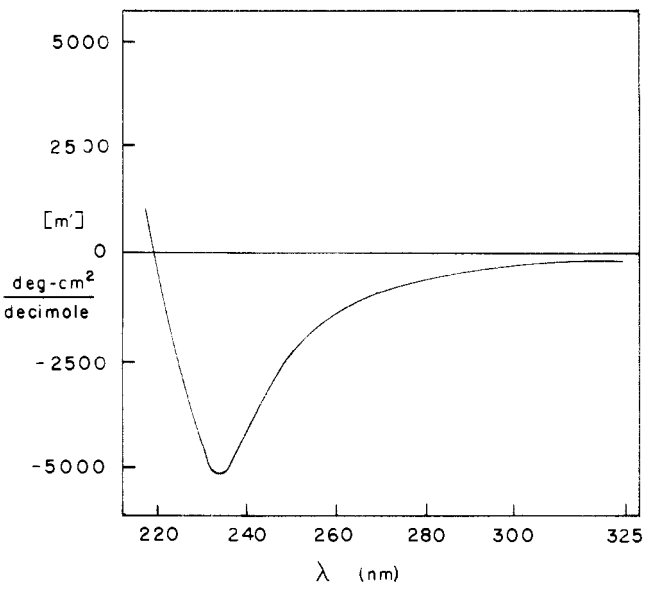

FIGURE 1: Optical rotatory dispersion of salt-extracted whole calf thymus histones under conditions: 0.5 mg/ml in 0.08 M NaCl, 0.02 M NaOAc, pH 5, and 20 °C.

histone is fractionated into H1–H4–H3 and H2a–H2b fractions by chromatography on Sephadex G-100. Fractions H1 and H4–H3 are prepared by 70% saturated $(NH_4)_2SO_4$ precipitation of H4–H3 from the first eluted chromatographic peak. Histone H2a is purified from whole histone by chromatography of a 1.5 ml, 7 mg/ml sample on a 130 × 1 cm column of Bio-Gel P-60 at 4 °C in 0.1 M NaCl, 0.02 M HCl, 0.1 mM PMSF, as in Böhm et al. (1973), except samples were not previously dissolved in urea before application. Histone purity was assessed by gel electrophoresis in 2.5 M urea, pH 2.7, according to the method of Panyim and Chalkley (1969). Whole histone appears free of protamine, and histone fractions H1, H4–H3, and H2a appear essentially pure. Fraction H2a–H2b contains additional minor staining bands.

Sedimentation velocity and equilibrium experiments were performed using a Beckman Model E analytical ultracentrifuge equipped with a Rayleigh interference optical system in which the camera lens is focused at the $\frac{2}{3}$ rds plane of a 12-mm cell. Sedimentation velocity experiments used a scribed capillary-type double-sector centerpiece to generate a synthetic boundary. Velocity experiments were performed at 56 000 rpm and 20 ± 0.1 °C with a protein concentration of 1.24 mg/ml. Photographs of interference fringe patterns were made at 16-min intervals until a concentration plateau was no longer present. Fringe patterns were analyzed by means of an automated microcomparator capable of estimating mean fringe positions (Carlisle et al., 1974). The weight-average sedimentation coefficient, $\bar{s}$, was determined by trapezoidal numerical integration, over the boundary of the fringe displacement, to determine the second moment position, r_z (Schachman, 1959). "Blank" experiments (both cell sectors contain water) were subtracted from each boundary photograph prior to integration. Error estimates for $\bar{s}$ were made from the least-squares analysis of $\ln r_z$ versus $r^2/2$.

High-speed sedimentation-equilibrium experiments were conducted according to the methods of Yphantis (1964) and Roark and Yphantis (1969). Experiments were carried out in 12-mm external-loading multichannel cells that permit blank pattern correction without disassembly. Some experiments used an argon multiplexed laser light source with photographs on Kodak IV F spectroscopic plates. Partial specific volumes

[1] Abbreviations used are: PMSF, phenylmethylsulfonyl fluoride; EDTA, (ethylenedinitrilo)tetraacetic acid.

TABLE I: Denaturation Susceptibility of Salt-Extracted Histones.[a]

Treatment	% Helix[b]	Δ%[c]	$\bar{s}$[d]	$\Delta\bar{s}$[e]
0.01 N HCl	22.5 ± 4.5	−9.2	1.81 ± 0.04 S	0.14 S
pH 3.0[f]	23.8 ± 3.1	−7.9	1.85 ± 0.03	0.18
pH 4.5[f]	26.7 ± 4.8	−5.0	1.79 ± 0.04	0.12
5 M urea[g]	34.1 ± 5.1		1.46 ± 0.02	−0.21
2.5 M urea[g]	34.6 ± 2.0		1.52 ± 0.01	−0.15
40% guanidine-HCl[h]	29.3 ± 2.5		1.45 ± 0.03	−0.22
pH 7.5[f]	29.6 ± 3.9		1.72 ± 0.07	
pH 9.0[f]	30.4 ± 3.7		1.61 ± 0.04	
pH 10.5[f]	33.5 ± 5.4		1.59 ± 0.06	
0.02 M HCl, 0.1 M NaCl	28.1 ± 3.7		1.68 ± 0.03	
0.25 N H_2SO_4	35.3 ± 2.0		1.71 ± 0.01	
0.4 M H_2SO_4–C_2H_5OH[i]	31.5 ± 3.5		1.65 ± 0.03	
C_2H_5OH–HCl[j]	27.6 ± 2.0		1.64 ± 0.03	
C_2H_5OH (95%)	29.8 ± 2.0		1.70 ± 0.09	
Acetone (95%)	31.2 ± 3.1		1.62 ± 0.05	
Acetone–HCl[k]	31.4 ± 2.0		1.66 ± 0.04	
10% guanidine-HCl	29.5 ± 2.8		1.70 ± 0.01	
20 M NaCl	31.3 ± 4.1		1.62 ± 0.02	
Untreated	31.7 ± 2.0		1.67 ± 0.02	

[a] Values of percent helix and $\bar{s}$ in reference solvent after exposure to treatment. Upper portion of table lists probable denaturing treatments. [b] Percent α helix from $[m']_{233}$. [c] Change in percent helix compared to no treatment. Only significant changes are given. [d] Weight-average sedimentation coefficient. [e] Change in $\bar{s}$ compared to no treatment. Only significant changes are given. [f] With 0.1 M histidine. [g] With 0.15 M NaCl. [h] With 0.13 M phosphate, pH 6.8. [i] Sample in 0.4 M H_2SO_4 dialyzed against 95% C_2H_5OH. [j] Four volumes of 95% C_2H_5OH:one volume of 1.25 M HCl. [k] Three volumes of acetone:one volume of 0.25 M HCl.

were estimated from the amino acid composition (Haschemeyer and Haschemeyer, 1973) and corrected for Donnan effect nonideality (Roark and Yphantis, 1971): H4–H3, 0.733 ml/g; H2a–H2b, 0.732 ml/g; H1, 0.748 ml/g; and H2a, 0.734 ml/g. Local weight average, M_w, and z average, M_z, molecular weights were estimated by a computer program developed by the author (D.E.R.).

The percent α helix was determined at room temperature by optical rotatory dispersion measured from 210 to 325 nm using a Cary 60 recording spectropolarimeter equipped with a 0.5-mm path length cell. The reduced mean residue rotation $[m']$ at 233 nm corresponds to the optical rotatory dispersion trough characteristic of α helices. Helix content was estimated by assuming a value of $[m']_{233}$ of −15 000 deg-cm^2/dmol for 100% α helix (van Holde, 1971) using a protein concentration of 0.5 mg/ml. This method could reliably detect changes of 3% helix. Error estimates for percent helix included error estimates in both rotation and concentration.

Histone concentrations were determined by the microbiuret method (Itzhaki and Gill, 1964) calibrated with lyophilized histones. Multiple determinations of concentration were used for optical rotatory dispersion studies.

Results

Denaturation Studies. Calf thymus histones are susceptible to denaturation by treatment with different solvent systems. Experiments are designed to detect only irreversible changes. Thus, identical solvent conditions (0.08 M NaCl, 0.02 M NaOAc, pH 5) are used in determining percent α helix and weight-average sedimentation coefficient $\bar{s}$, before and after treatment with the perturbing solvent. After measurement of $[m']_{233}$ and $\bar{s}$ for the whole histone solution in its native state, the solution is dialyzed against the appropriate treatment buffer at 4 °C for 12 h. Samples are then exhaustively redialyzed against the reference buffer; and the $[m']_{233}$ and $\bar{s}$ measurements are repeated. In all cases, the protein concentration is adjusted to be identical before and after treatment. Synthetic boundary formation is necessary for accurate sedimentation measurements due to rapid diffusion, which would otherwise prevent significant separation of the boundary from the meniscus. Figure 1 presents a partial optical rotatory dispersion spectra of untreated whole histone in the reference solvent. The trough at 233 nm indicates the absence of β structure and an α-helical content of 32 ± 2%. The $\bar{s}$ of untreated whole histone is 1.67 ± 0.02 S, with no evidence of rapidly sedimenting protein. Table I presents percent helix and $\bar{s}$ for a series of treatments. Those treatments resulting in irreversible denaturation appear at the top of the table. Denaturation is considered demonstrated if one or both parameters change by at least two standard deviations. Low pH or treatment with urea or guanidine (40%) produces irreversible denaturation. Decreasing pH produces a progressive decrease in percent helix and an increase in the tendency to aggregate as measured by $\bar{s}$. Increasing urea concentrations have the opposite effect: increased helicity and decreased $\bar{s}$. Not all low pH conditions produce irreversible denaturation. For instance, while 0.01 and 0.02 N HCl denature, addition of 0.1 M NaCl prevents the denaturation, probably by suppressing ionic repulsive interactions. Histone sulfates are less easily denatured than the histone chlorides. Histone is precipitated in the presence of ethanol or acetone, but is not irreversibly denatured.

Histone Interactions. Fractions H4–H3 and H1 are prepared from the rapidly eluting peak on Sephadex G-100 chromatography of the whole histone. Separate chromatography of the H4–H3 fraction and the H1 fraction gave elution at identical positions to that of the original peak. This indicates a probable absence of interaction between the H4–H3 complex and H1.

Sedimentation-equilibrium experiments were performed on the H4–H3 fraction using a lower ionic strength (0.01 M NaOAc, pH 5) than in our previous studies (Roark et al., 1974;

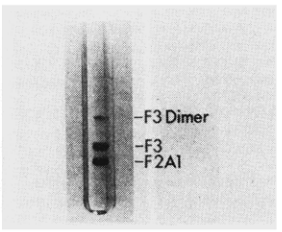

FIGURE 2: Gel electrophoresis of H4–H3 fraction previously dialyzed against 0.01 M NaOAc, pH 5. Electrophoresis performed according to Panyim and Chalkley (1969).

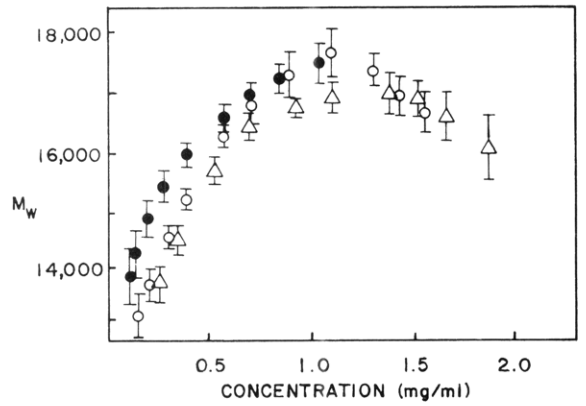

FIGURE 3: High speed sedimentation-equilibrium experiment for H4–H3 in 0.01 M NaOAc, pH 5, at 20 °C and 48 000 rpm. Weight-average molecular weight is presented for three initial loading concentrations: (●) 0.25 mg/ml, (○) 0.5 mg/ml, and (△) 1.0 mg/ml.

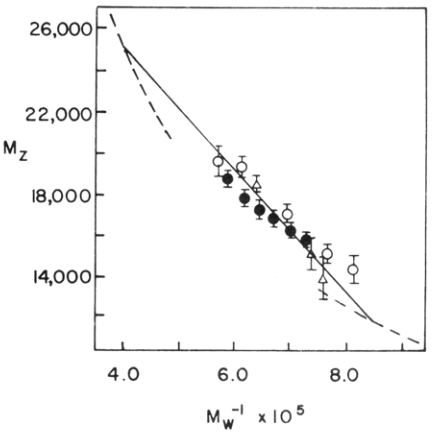

FIGURE 4: "Two-species plot" for sedimentation equilibrium of H4–H3 in 0.01 M NaOAc, pH 5. The conditions are as in Figure 3. Dashed line represents the hyperbola $M_z\,(1/M_w) = 1$. Intersections of solid "two-species" line with hyperbola estimates molecular weights of the two species.

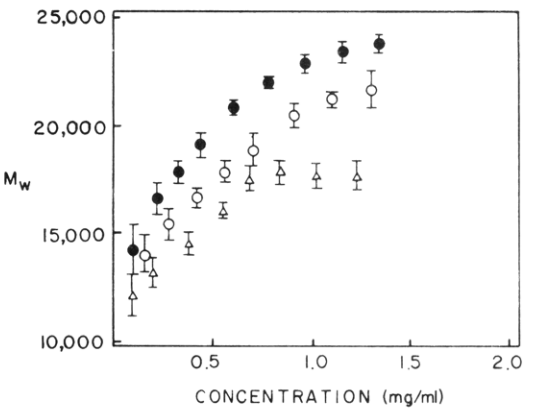

FIGURE 5: High speed sedimentation-equilibrium experiment for H2a–H2b fraction in 0.05 M NaOAc, 0.05 M $NaHSO_3$, 0.01 M EDTA, pH 5. Other conditions are those of Figure 3, except that the initial loading concentrations are: (●) 0.33 mg/ml, (○) 0.75 mg/ml, and (△) 1.5 mg/ml.

Roark, 1976). These experiments are complicated by the appearance of small amounts of an H3 dimer (see Figure 2). Low ionic strength may result in the unmasking of a previously inaccessible cysteine group of H3, possibly by dissociation of the H4–H3 complex (see below). Figure 3 presents the results of a high speed sedimentation-equilibrium experiment performed at 20 °C and 48 000 rpm for the low ionic strength condition. Downward deviation of the weight-average molecular weight, M_w, at high concentrations is due to Donnan nonideality (Roark and Yphantis, 1971). Figure 4 presents the data in terms of a "two-species plot" (Roark and Yphantis, 1969). Extrapolation of the data to the hyperbola, $M_z\,(1/M_w) = 1$, indicates the presence of two molecular weight species with approximate molecular weights of 13 000 and 23 500, corresponding to histone monomers and dimer. We conclude that under low ionic strengths, the (H4)(H3) dimer exists in equilibrium with the separate monomers; and that the $(H4)_2(H3)_2$ tetramer is not significantly present.

High speed sedimentation-equilibrium studies on the H2a–H2b fraction are presented in Figures 5 and 6. The lack of overlap of the M_w vs. C curves for different initial loading concentrations indicates heterogeneity of the thermodynamic components. The "two-species" analysis indicates a species with an approximate molecular weight of 12 000 and a larger species with a molecular weight near 30 000. This is consistent with separate histone monomers (average mol wt 13 900) and an (H2a)(H2b) dimer with a molecular weight of 28 000. The heterogeneity could be due to histone with different association constants, or to competing self-association of either or both histones.

Sedimentation-equilibrium experiments on histone H1 were performed at two ionic strengths. Figure 7 presents data from an experiment at 36 000 rpm, 20 °C in 0.08 M NaCl, 0.02 M NaOAc, pH 5. The "two-species plot" indicates that most material is in the monomeric state, molecular weight near 20 000, along with some dimer. An experiment at 1 M NaCl, 0.02 M NaOAc, pH 5, demonstrated that, under these conditions, only the monomer is present. Fraction H1 evidently weakly associates to the dimer at ionic strengths near 0.1, pH 5; this association is suppressed by increased ionic strength.

The self-association of fraction H2a is more complex than that of H1. Figure 8 is a presentation of the weight-average molecular weight as a function of concentration for a high-speed sedimentation-equilibrium experiment at 36 000 rpm, 20 °C, in 0.2 M NaCl, 0.02 M NaOAc, pH 5. The H2a readily associates to polymers larger than a tetramer of the 14 000 molecular weight monomer. Figure 9 presents a "two-species plot" for this experiment. Curvature of the two-species line demonstrates the presence of more than two species. Extrapolation of the two-species line to the hyperbola $(M_z)(1/M_w) = 1$ permits estimation of the smallest and largest predominant species. Assuming only multimers of the 14 000 molecular weight species are present, the two-species analysis indicates that both the 28 000 molecular weight dimer and the 112 000 molecular weight octamer are present. The curvature of the plot can be accounted for by assuming the additional presence of the 56 000 molecular weight tetramer. Least-squares analysis of the equilibrium concentration distribution as a function of radial position demonstrates adequate fit of the data if the three species (dimer, tetramer, and octamer) are

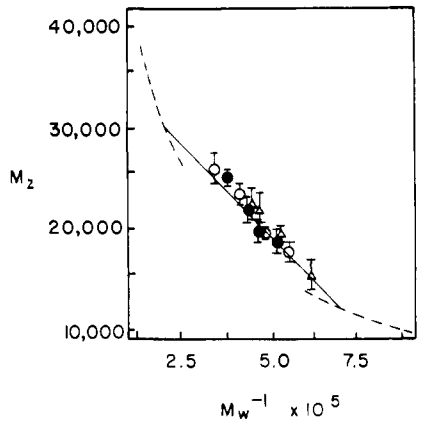

FIGURE 6: "Two-species plot" for sedimentation-equilibrium experiment of Figure 5 on histones H2a–H2b. Solid and dashed lines are two-species line and $M_z(1/M_w)$ hyperbola, respectively.

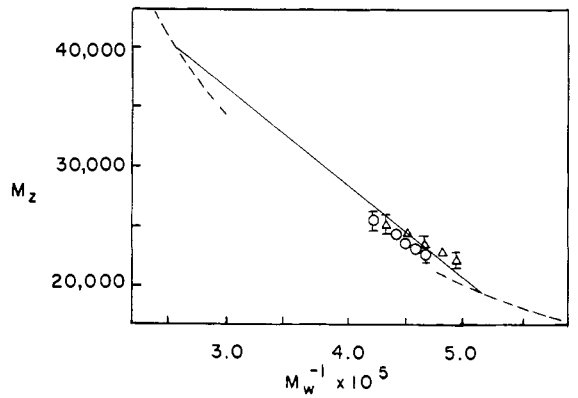

FIGURE 7: Sedimentation-equilibrium "two-species plot" presenting the association behavior of histone H1. Experimental conditions are 0.08 M NaCl, 0.02 M NaOAc, pH 5; 20 °C and 36 000 rpm. Initial loading concentrations are: (○) 1.0 mg/ml and (△) 2.0 mg/ml.

assumed present. The variance is 5 μm of interference fringe displacement (0.005 mg/ml). If only dimer and octamer are assumed, the variance increases to 13 μm. The simplest model to explain the H2a self-association is that of sequential association of the dimer to tetramer and octamer. However, more complex models involving additional intermediate species such as hexamers have not been eliminated. Assuming the dimer-tetramer-octamer model, the least-squares analysis of the sedimentation-equilibrium data indicates that the predominant species is the 56 000 molecular weight tetramer, over the concentration range 0.1–1.0 mg/ml. For this same concentration range, the predominant H1 species is the monomer.

Conformation of H4–H3 Tetramer. Weight-average sedimentation coefficients were estimated by the second moment technique to investigate the molecular size of the $(H4)_2(H3)_2$ tetramer. Values of $s_{20,w}$ decreased with decreasing salt concentration, possibly indicative of charge effect nonideality. The primary charge effect should be essentially suppressed with 0.5 M NaCl, 0.05 M NaOAc, pH 5. An initial loading concentration of 2.8 mg/ml, sufficient to promote essentially complete association to tetramer, yielded a $s_{20,w}$ of 2.53 S. The secondary charge effect is not suppressed by increased ionic strength, but values of $s_{20,w}$ may be corrected for this effect (Eisenberg, 1976). This correction yields an $s_{20,w}$ of 2.81 S for the 54 000 molecular weight tetramer. From knowledge of the $s_{20,w}$ and the molecular weight, the frictional ratio f/f_0 is estimated to be 1.63. The ratio may be factored into hydration and asymmetry terms. Kuntz (1971) has described a method

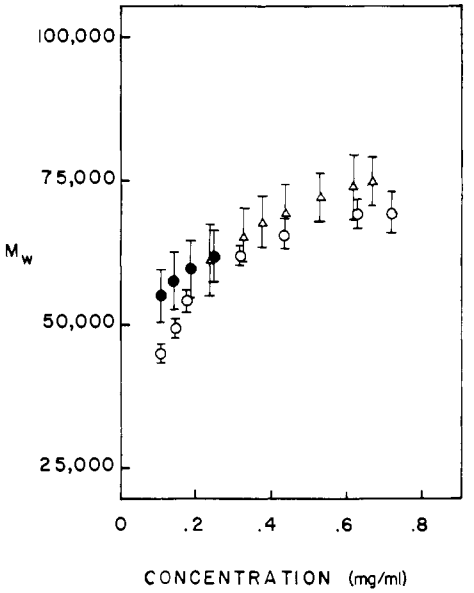

FIGURE 8: Association behavior of H2a. High speed sedimentation-equilibrium conditions are: 0.2 M NaCl, 0.02 M NaOAc, pH 5 at 36 000 rpm. Initial loading concentrations are (○) 0.5 mg/ml, (△) 0.25 mg/ml, and (●) 0.125 mg/ml.

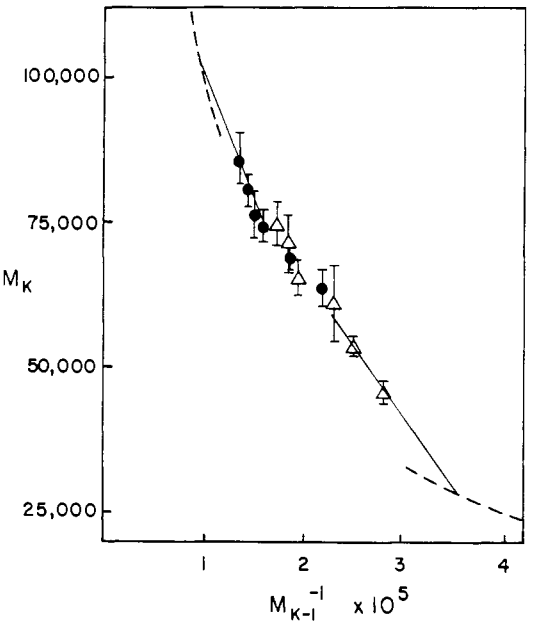

FIGURE 9: "Two-species plot" of H2a association for sedimentation-equilibrium experiment of Figure 8. Solid and dashed lines are two-species line and hyperbola, $M_k(1/M_{k-1}) = 1$, respectively. (●) corresponds to M_z vs. $1/M_w$ and (△) is M_w vs. $1/M_n$. (For systems containing two species, the two plots are coincident.)

for estimating protein hydration from amino acid composition analogous to that used to estimate partial specific volumes. The method assumes all amino acids are fully hydrated, and is, thus, an overestimation. In practice, the error is small, since hydrophobic groups hydrate much less than polar groups. The calculated hydration for the H4–H3 complex is 0.34 g of H_2O/g of protein. This results in an asymmetry factor $(f/f_0)_{asy}$ of 1.44. (Failure to correct for charge effects or hydration would yield an even greater ratio.) If an equivalent prolated ellipsoid is assumed representative of the tetramer, the axial ratio calculated from this frictional ratio is approximately 8. This high asymmetry is perhaps surprising for a protein of

TABLE II: Effect of Dextran on Apparent Molecular Weight of Histones.

Fraction	Solvent	Column[a]	K_{av}[b]	$M_{apparent}$
H4-H3	0.01 M NaOAc, pH 5.0	Sephadex G-75	0.091	100 000
H4-H3	0.01 M NaOAc, pH 5.0	Bio-Gel P-60	0.109	42 500
H4-H3	0.01 M NaOAc, pH 5.0, 0.5% dextran	Bio-Gel P-60	0	>60 000
H4-H3	0.01 M NaOAc, pH 5.0, 0.5% glucose	Bio-Gel P-60	0.097	44 000
H1	0.01 M NaOAc, pH 5.0	Bio-Gel P-60	0.074	48 500
H1	0.01 M NaOAc, pH 5.0, 0.5% dextran	Bio-Gel P-60	0	>60 000
H2a-H2b	0.01 M NaOAc, pH 5.0	Bio-Gel P-60	0.198	33 500
H2a-H2b	0.01 M NaOAc, pH 5.0, 0.5% dextran	Bio-Gel P-60	0.123	40 500

[a] Columns used are 1.1 × 45 cm Sephadex G-75 and 0.9 × 90 cm Bio-Gel P-60. [b] $K_{av} = (V_e - V_v)/(V_t - V_v)$, where V_e, V_v, and V_t are elution, void, and total column volumes, respectively.

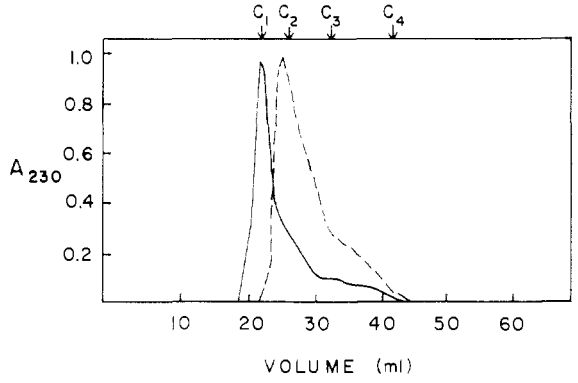

FIGURE 10: Chromatography of H4-H3 fraction on Bio-Gel P-60. Solid curve is elution profile for histone in 0.01 M NaOAc, pH 5 plus 0.5% dextran (T-500, Pharmacia). Dashed curve is as above, but without dextran. Column calibrating markers C1, 2, 3, and 4 correspond to elution positions of Blue Dextran (Pharmacia), ovalbumin, chymotrypsin, and ribonuclease, which elute independently of dextran concentration.

moderate α-helical content. The percent α helix, determined by optical rotation at 233 nm, is 28 ± 4%.

Histone Behavior in the Presence of Dextrans. Histone fractions have anomalously high apparent molecular weights on the cross-linked dextran gel Sephadex. Comparison between apparent molecular weights on calibrated Sephadex G-75 and Bio-Gel P-60 columns is shown in Table II. Under the conditions, 0.01 M NaOAc, pH 5, the apparent molecular weight of the H4-H3 fraction is 100 000 on G-75, but only 42 500 on P-60. Figure 10 shows that if 0.5% soluble dextran (T-500, Pharmacia, M_w = 500 000) is additionally used in the chromatography of the H4-H3 fraction on P-60, the fraction chromatographs at the void volume with an apparent molecular weight greater than 60 000. A similar effect is observed for histone H1, and a small effect for H2a-H2b. No effect of soluble dextran is observed on the column calibrating proteins, ovalbumin, chymotrypsin, or ribonuclease. Since the anomalous behavior occurs with both the immobolized dextran of Sephadex and the soluble dextran on Bio-Gel, but not on Bio-Gel alone, the dextran is responsible for the higher apparent molecular weights. Chromatography on P-60 in the presence of the monosaccharide glucose gave no anomaly.

Discussion

Determination of protein secondary structure and mean aggregating characteristics by measurement of $[m']_{233}$ and $\bar{s}$ in a reference solvent indicates that the treatments appearing in the upper portion of Table I may cause irreversible denaturation. These treatments probably should be avoided in histone fractionations if the histones are later to be used for interaction, structural, or reconstitution studies. Of course, failure to detect a significant change in the two monitored parameters does not prove the absence of denaturation due to other treatments. Several previous histone interaction studies have employed chromatography with elution by 0.01 or 0.02 N HCl, which we have shown to produce irreversible conformational change with increased aggregation. Thus, the reported self-assembly of H4 (Sperling and Bustin, 1974); and the formation of H4-H2b and H2a-H2b complexes (D'Anna and Isenberg, 1973, 1974b) should be reexamined using histones prepared by other means. The recent interaction study of both single histones and histone pairs (Sperling and Bustin, 1975) used histone redissolved in 0.01 N HCl, after lyophilization, and preparation of H2a and H3 by a method involving exposure to 4 M urea (Ruiz-Carrillo and Allfrey, 1973). These treatments and, possibly, the higher pH of their studies, may account for their observations of larger aggregates than those reported here. D'Anna and Isenberg (1974a) have repeated the studies of the H4-H3 complex (Roark et al., 1974; Kornberg and Thomas, 1974) using H4 prepared by 0.01 N HCl chromatography and have verified the existence of the $(H4)_2(H3)_2$ tetramer. Their data does show, however, stronger association to the tetramer with an absence of equilibrium with the dimer. The studies of Kelly (1973) did not employ procedures we have found to be denaturing, and indicate the existence in solution of a 1:1 complex of histones H2a-H2b. This complex has also been reported by others (Kornberg and Thomas, 1974; D'Anna and Isenberg, 1974b; Skandrani et al., 1972). The popular fractionation procedures of Johns (1964, 1967) employ exposure to guanidine and to 0.25 N HCl, both of which may result in denaturation. Thus, the aggregation studies of Edwards and Shooter (1970) and Diggle et al. (1975), which made use of these histone preparations, need cautious interpretation.

Kelly (1973) observed the (H2a)(H2b) dimer by sedimentation equilibrium without detecting histone monomer or heterogeneity. His lower speed experiments would not be sensitive to heterogeneity, and the concentrations he employed are such that our data would also have indicated predominantly dimer. We conclude that our results are consistent with those of Kelly, and that dissociation of the (H2a)(H2b) dimer probably occurs at low concentrations. The predominant cross-interactions in solution demonstrated thus far with presumably nondenatured histones are the (H4)(H3) dimer and tetramer and an (H2a)-(H2b) dimer.

The $(H4)_2(H3)_2$ tetramer has a helical content typical of globular proteins. Yet the axial ratio, assuming an equivalent compact prolate ellipsoid, is 8. An alternative model to this

highly asymmetric prolate is a structure with a compact semispherical center and several polypeptide chains protruding freely into solution. Both H4 and H3 have charged groups clustered in the amino-terminal region, while the hydrophobic groups are predominantly in the carboxyl-terminal region. Sung and Dixon (1970) have proposed that the amino-terminal region of H4 may become α helical to conform to the major groove of the DNA with favorable ionic interactions between positive charges on the histone and negative charged phosphate groups of the DNA. Nuclear magnetic resonance studies have suggested that the amino-terminal region of H4 in solutions is flexible (Pekary et al., 1975). Thus, the tetramer, $(H4)_2(H3)_2$, may have a compact core consisting of the carboxyl-terminal regions of the four subunits, and four protruding amino-terminal regions capable of interacting with DNA. Such a model could explain the high frictional coefficient, and would be ideal for compacting chromatin by interacting with DNA at four loci.

The dextran effect on the apparent molecular weights of histone fractions H1 and H4–H3 is puzzling. The effect is not that of simple interaction between the protein and the dextran gel, since such interactions would retard elution and yield an apparently smaller molecular weight. The specificity of this effect to type of polysaccharide is not known. Since elution displacement did not occur for the calibrating proteins upon addition of soluble dextran to the P-60 chromatography, the effect appears to be peculiar to histones. The cause may be one of mere entanglement of polysaccharide and histone chains that does not occur either with the calibrating proteins or with histone and the polyacrylamide Bio-Gel; or the cause may involve some physiologically useful histone aggregation promoted by polysaccharides of the nucleus. Studies to determine the aggregation state of histone in the presence of polysaccharide are needed to explore this problem.

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