Configuration of Unsheared Nucleohistone. Effects of Ionic Strength and of Histone F1 Removal†

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ABSTRACT: Nucleohistone solubilized from rabbit thymus nuclei by an endogenous nuclease has in 0.15 *M* salt an exceptionally low intrinsic viscosity and very high sedimentation velocity. A fully reversible expansion of configuration occurs on lowering ionic strength. When $[\eta]$ is plotted against $I^{-1/2}$ and extrapolated to high I, $[\eta] = 0$ is reached at I = 0.4–1 *M* and $[\eta]$ at $I = \infty$ is negative, contrary to the behavior of DNA and of the great majority of polyelectrolytes, which extrapolate to a positive $[\eta]$ at $I = \infty$. This behavior demands that the configuration of nucleohistone depends not only on electrostatic expansive forces but also on contracting forces which are not electrostatic and do not go to zero in any accessible configuration. Intramolecular hydrophobic bonds might provide such contracting forces. Increasing I above 0.15 *M* leads to precipitation near 0.3 *M* and redissolution with dissociation of F1 and expansion in 0.6 *M*. The expansion is largely but not completely reversed on return to 0.15 *M*. Much further expansion occurs in I = 1.2 *M*. Nucleohistone exposed to 1.2 *M* could not be redissolved in the original medium. Nucleohistone depleted of F1 exhibits a similar expansion as ionic strength is reduced, at higher viscosities throughout. On extrapolation to $I = \infty$ both positive and negative viscosities were observed, on different lots, perhaps reflecting variable extraction of other histones. Circular dichroism spectra are very little affected by ionic strength (0.6 *M* and lower) or F1 removal, despite tenfold changes in viscosity.

Chromatin in the interphase nucleus consists of enormous molecules, and soluble particles of a size amenable to hydrodynamic experiments must be obtained by cleavage. Shearing in a blender has been the most common means of fragmentation. In such shearing processes covalent bonds are broken by tensile forces arising from fluid friction. Since the DNA in chromatin is coiled or contorted, constrained by associated proteins, it is plausible that it should be elongated by tensile forces, and, further, rearrangements of the bound proteins may occur under strain that are not wholly reversible. That such damage occurs was shown recently by Noll et al. (1975), who found that shearing destroys the specificity of nuclease cleavage, and also indicated by our earlier report (Rees et al., 1974a) that the large nucleohistone particles obtained from thymus nuclei by the limited action of an endogenous nuclease have properties quite distinct from nucleohistone solubilized by shearing, and, further, can be precipitated by shearing.

This unsheared nucleohistone lends itself particularly well to studies of the configuration of nucleohistone, because damage by shearing and pelleting has been avoided, also because it exhibits the condensed state in extreme degree, and because it is freely soluble in 0.15 *M* buffers, in which most chromatin preparations are precipitated. To obtain these advantages a minor degree of histone proteolysis, which we could not inhibit, has been accepted, and we have proceeded with experiments on the effects of high and low ionic strength and of histone F1 removal on its sedimentation, viscosity, and circular dichroism.

Its properties in the 0.15 *M* buffer in which it is prepared and fractionated, and the properties of the DNA isolated from it, have been described (Rees et al., 1974a). DNA of several million molecular weight and of a normal proportion of repetitive and unique sequences is contained in nucleohistone of very high sedimentation velocity and very low intrinsic viscosity. The five histones are present in nearly normal amounts with two additional bands taken to be specific cleavage products. Sepharose chromatography is used to obtain fractions of limited heterogeneity. The pooled fractions examined in the present experiments typically constituted about 25% of the nuclear DNA.

Our present results show a striking contrast between this nucleohistone and nearly all other flexible polyelectrolytes, including DNA and sheared nucleohistone, in the ionic strength dependence of intrinsic viscosity. $[\eta]$ of DNA and most polyelectrolytes when extrapolated on a reciprocal plot to infinite I has a positive value. This $[\eta]_\infty$ may be taken to reflect the configuration the polyelectrolyte would have if unperturbed by electrostatic forces. $[\eta]$ of this nucleohistone, however, extrapolates to zero $[\eta]$ at a finite I and to negative values of $[\eta]_\infty$.

We offer the interpretation that the configuration of nucleohistone depends on the balance between electrostatic repulsive forces and intramolecular contracting forces which are not ionic strength dependent, and very probably are hydrophobic in origin.

Materials and Methods

The nucleohistone was prepared from rabbit thymus nuclei by the action of a magnesium-dependent endogenous nuclease essentially as described by Rees and Krueger (1968) with the modifications of Rees et al. (1974a). Particular care was taken to minimize agitation of the reaction mixtures. In order to obtain a high molecular weight product, the reactions were stopped when 20–50% of the nuclear DNA was solubilized. Higher solubilization is correlated with lower molecular weight. The solubilized nucleohistone

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Table I: Typical Properties of Soluble Nucleohistone in 0.1 *M* KCl–0.02 *M* Cacodylate–0.005 *M* Citrate (pH 7.4), $I = 0.15$ *M*.

Expt	A	B
Nucleohistone		
$s_{20,w}^0$	135	146
$[\eta]$, dl/g of DNA	0.51	0.77
$[\eta]$, dl/g of NH[a]	0.22	0.34
$M \times 10^{-6}$ [b]	18.0	25.2
DNA Isolated from NH		
$s_{20,w}^0$ [c]	19.6	19.6
$[\eta]$, dl/g[d]	44.0	44.0
$M \times 10^{-6}$ [d]	7.03	7.03
Ratios		
M_{NH}/M_{DNA}	2.6	3.6
$[\eta]_{DNA}/[\eta]_{NH(DNA)}$	86.0	57.0

[a] Calculated on the basis of DNA/protein = 1:1.3. [b] Calculated using the equation of Scheraga and Mandelkern (1953) with $\beta = 2.11 \times 10^6$ and $\bar{v} = 0.665$ (estimated from composition). Since the particles are elongated or irregular, these molecular weights are upper limits. The calculation is insensitive to shape and these molecular weights are unlikely to be more than 20% high. [c] Corrected to zero concentration by the equation of Eigner (1968). [d] Calculated from sedimentation velocities using the equations of Crothers and Zimm (1965).

was chromatographed and studied, unless otherwise noted, in a buffer which is 0.1 *M* KCl–0.02 *M* sodium cacodylate–0.005 *M* citric acid, made pH 7.4 with HCl, and ionic strength 0.15 *M*. The nucleohistone was purified and fractionated on Sepharose 2B. About 30 ml of reaction mixture supernatant was applied to a 2.5 × 45 cm column which was run at 4°C with an upward flow rate of 3.5 ml/hr. Fractions from the major peak of the chromatogram were pooled for further study. These usually contained about 60% of the DNA applied to the column.

The nucleohistone was dialyzed into both higher and lower ionic strength media. The high ionic strength buffers were concentrations of the original 0.15 *M* elution buffer while the lower ionic strengths were dilutions. The dialysis was done exhaustively in the cold (4°C) for 24 hr with mild agitation of the dialysate only. Dialyzed samples were clarified by centrifugation in the International 269 rotor at 2300 rpm for 10 min at 4°C. Recovery of A_{260} was 90% or better.

Histone fraction F1 was removed from the nucleohistone by first dialyzing pooled nucleohistone fractions into a 0.5 *M* ionic strength buffer followed by gel filtration chromatography on Sepharose 2B, eluting with the same 0.5 *M* buffer. About 20 ml of nucleohistone pool was applied to a 1.6 × 45 cm column which was run at 4°C with an upward flow rate of 3.5 ml/hr. The F1-depleted nucleohistone elutes at the column void volume. Fractions of low concentration were discarded and those containing the bulk of the A_{260} were pooled. The nucleohistone with histone F1 removed was dialyzed back into the original 0.15 *M* buffer and also to lower ionic strengths for physical studies.

DNA analyses were done by the method of Burton (1959), or in the case of nucleohistone by $A_{260\,nm}$ using an extinction coefficient of 24 cm^2/mg (Rees et al. 1974a). Protein assays were done according to Lowry et al. (1951) and RNA was determined by the orcinol reaction according to Schneider (1957). Histones extracted into 0.4 *M* H_2SO_4 were precipitated with cold ethanol, redissolved in 0.9 *M* acetic acid, and subjected to gel electrophoresis according to Panyim and Chalkley (1969).

DNA was prepared for circular dichroism experiments by a variant of the method of Huang and Bonner (1962), and for sedimentation and viscosity measurement DNA was isolated from nucleohistone particles by banding in CsCl and handled in a manner to avoid shear.

Sedimentation rates were measured by boundary sedimentation at 20°C in a Beckman Model E analytical ultracentrifuge with photographic ultraviolet absorption optics. To avoid shear the cells were filled using an 18-gauge needle. Convection was avoided with a sucrose gradient generated by diffusion (suggested by Dr. V. Schumaker). Films were scanned using the Gilford spectrophotometer with film densitometer described by Rees (1971).

Viscometry was done at 20 ± 0.01°C in a rotating cylinder viscometer similar to that described by Gill and Thompson (1967) and operated at shear rates of less than 0.5 sec^{-1}. η_{sp}/c was independent of concentration and of shear rate throughout the range of measurements (Rees et al., 1974a, and data not shown).

CD spectra were obtained with a Cary Model 61 circular dichrometer with on-line computer processing of data by a Cary Spectrosystem 100. Spectra were averages of 90 readings at each wavelength. Experiments were done in 1-cm path length cells at 20°C. Sample concentrations were determined by $A_{260\,nm}$ using extinction coefficients, ϵ_p, of 6600 M^{-1} cm^{-1} for DNA and 7920 M^{-1} cm^{-1} for the nucleohistone. The samples were not visibly turbid and their A_{320} was not measurable.

Results

Typical physical properties of the nucleohistone in a 0.15 *M* ionic strength buffer and of the DNA isolated therefrom are presented in Table I. These data from two experiments are from single fractions at the peak of the chromatogram. s, $[\eta]$, and M both of nucleohistone and DNA decline from early to late eluting fractions (Rees et al., 1974a). The nucleohistone exhibits an extreme degree of condensation of its DNA moiety as shown by its high sedimentation constant and very low intrinsic viscosity. The most dramatic illustration of the extent of condensation of the DNA is the ratio of DNA viscosity to nucleohistone viscosity based on its DNA complement. In one case (a) a condensation of 86-fold is seen while in the other preparation (b) a 57-fold reduction of the DNA viscosity is seen. These ratios are higher than reported previously (Rees et al., 1974a) indicating a more compact and perhaps more nearly native nucleohistone preparation. This results from minimizing agitation of the incubation mixture and we now routinely prepare nucleohistone with intrinsic viscosities of less than 0.3 dl/g. This must mean that the nucleohistone contains its DNA in a highly folded or very tightly coiled state. The molecular weight ratios indicate that some nucleohistone particles contain more than one DNA molecule, however, this is probably a result of the endonuclease action and not a relic of the native state.

The nucleohistone contains by mass 1 part DNA to 1.1 part histone to 0.2 part non-histone protein and no detectable RNA. Typical histone electrophoresis gel tracings are shown in Figure 1B and F and may be compared with histones from nuclei not incubated (Figure 1A). One additional band leading F1 is apparent, presumably the product of a specific proteolysis. Formation of this band was not inhibited by sodium bisulfite, diisopropyl fluorophosphate, or phenylmethanesulfonyl fluoride. The last at high concentration (2 and 10 mM) inhibited solubilization.

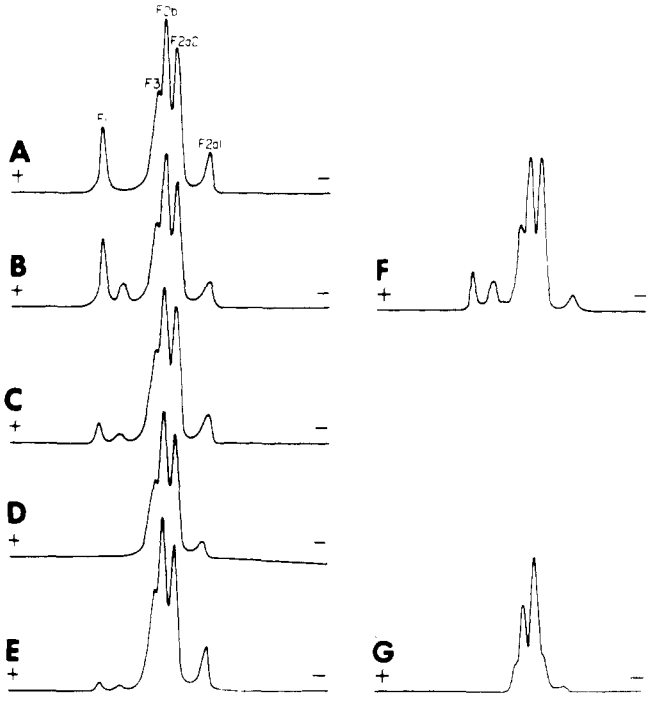

FIGURE 1: Tracings of gel electrophoresis of histones. (A) Extracted from saline-washed nuclei; (B and F) from soluble nucleohistone pelleted from 0.15 *M* medium by centrifugation 20 hr at 45000 rpm at 4°; (C) from nucleohistone pelleted from 0.5 *M* medium; (D) from nucleohistone after chromatography in 0.5 *M* medium, pelleted from 0.5 *M* medium; (E and G) from nucleohistone after chromatography in 0.5 *M* medium, dialyzed into and pelleted from 0.15 *M* medium. B through E represent one preparation, F and G, another.

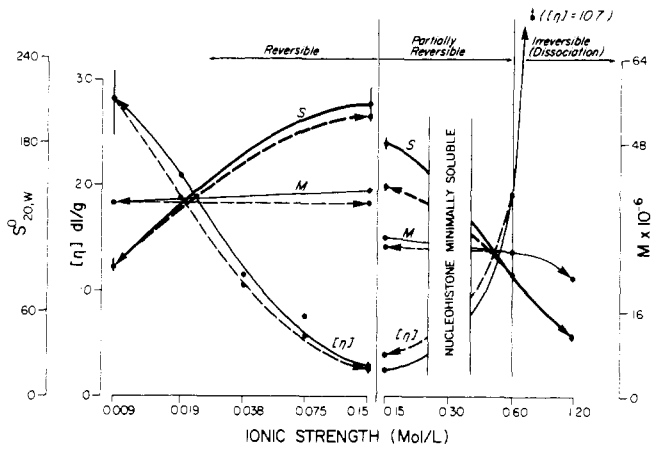

FIGURE 2: Hydrodynamic properties of nucleohistone as a function of ionic strength. One preparation shown was diluted or dialyzed from 0.15 *M* into various lower *I* media and was then dialyzed from 0.009 *M* back to 0.15 *M*. A second preparation was dialyzed from 0.15 *M* to 0.6 *M* or to 1.2 *M*. Part of this was dialyzed from 0.6 *M* back to 0.15 *M*. *M* was calculated from *s* and [η]. The shape of the curves for *s* which connect only two data points has been inferred from the viscosity data assuming constancy of *M*. The particular values of *I* are a binary series.

Ionic Strength Experiments on Complete Nucleohistone. Figure 2 presents a plot of sedimentation constant, intrinsic viscosity, and molecular weight of the nucleohistone vs. ionic strength. The left part of Figure 2 presents the effects of placing the nucleohistone in lower ionic strength media while the right shows the results of raising the ionic strength. The data presented here are from one typical low ionic strength experiment and one high ionic strength experiment with two different nucleohistone preparations used. The solid lines with arrow heads at the ionic strength extremes indicate data taken as the ionic strength change (by dialysis or in some instances by dilution) was directed away from 0.15 *M* while the dashed lines indicate data taken as the ionic strength was directed back toward the original 0.15 *M*.

When the ionic strength was lowered to 0.009 *M*, the viscosity rose from 0.28 to 2.8 dl/g, a tenfold increase, while *s* decreased from 209 to 92, and the molecular weight remained essentially constant. On return to 0.15 *M* the original sedimentation constant and intrinsic viscosity were recovered within experimental error.

On raising the ionic strength the nucleohistone went through a region of precipitation in the vicinity of 0.3 *M*. In 0.6 *M* it redissolved and the viscosity rose to 1.9 dl/g, an eightfold increase over the value in 0.15 *M*, the sedimentation constant fell to about ½ the original value, while *M* was again relatively unchanged. On lowering the ionic strength back to 0.15 *M* the nucleohistone approached its original configuration but remained somewhat extended (*s* was significantly lower than original *s* in 0.15 *M*). Gel electrophoresis verified that in 0.6 *M* histone F1 was quite dissociated whereas the other histones were pelleted with the DNA in normal amounts.

When the nucleohistone was dialyzed into 1.2 *M* medium the viscosity rose about 45-fold to a value of 10.7 dl/g, the sedimentation constant fell to 42, and the molecular weight calculated from *s* and [η] fell about 20%. Attempts to return this dissociated nucleohistone to the original buffer resulted in complete precipitation.

The viscosities measured at 0.15 *M* and lower I on five preparations are shown in Figure 3 plotted against the reciprocal square root of ionic strength. Plotting against $I^{-1/2}$ linearizes the ionic strength dependence of viscosity of DNA and many other flexible polyelectrolytes (see Discussion). The present plots are linear within experimental error. Excepting one case at 0.15 *M* only, where the data converge, the viscosities of these preparations fall in the same order as their molecular weights. Extrapolated to higher I, the viscosities fall to zero at I = 0.4 *M* to 1 *M* and all are negative at infinite I.

Preparation of F1-Depleted Nucleohistone. The conditions of partial dehistonization by gel chromatography lead to redistribution of histones from early to late eluting fractions, however, this redistribution is minimal in 0.5 *M* salt (Labourdette et al., 1974), and, further, on pooling fractions reequilibration occurs restoring homogeneity in composition (Labourdette et al., 1974).

The results of partial dehistonization in 0.5 *M* medium are shown in Figure 1. These gel electrophoresis patterns are of histones extracted from: (A) salt washed nuclei (included here for comparison); (B) nucleohistone pelleted from 0.15 *M*; (C) nucleohistone pelleted from 0.5 *M*; (D) nucleohistone after chromatography in 0.5 *M* and pelleted from 0.5 *M*; and (E) nucleohistone after chromatography in 0.5 *M*, dialyzed into 0.15 *M*, and pelleted.

Gels B–E represent one lot of nucleohistone and gels D and E are typical results of our conditions. Chromatography in 0.6 *M* (rather than 0.5) was found to cause partial depletion of histones other than F1 although pelleting from 0.6 *M* did not. Application of 25 rather than 20 ml to this column caused incomplete separation of F1. Excessive depletion of other histones occurred at times perhaps as a result of variation in nucleohistone molecular weight and con-

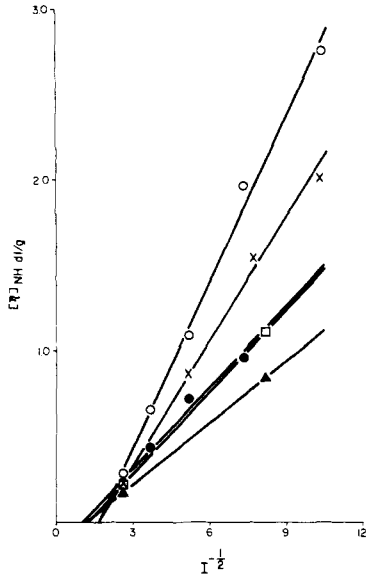

FIGURE 3: Intrinsic viscosity of nucleohistone at 0.15 *M* and lower ionic strengths plotted against the reciprocal square root of ionic strength. Molecular weights of these preparations were: (○) 39 × 10^6; (×) 30 × 10^6; (●) 20 × 10^6; (□) 18 × 10^6; (▲) 12 × 10^6.

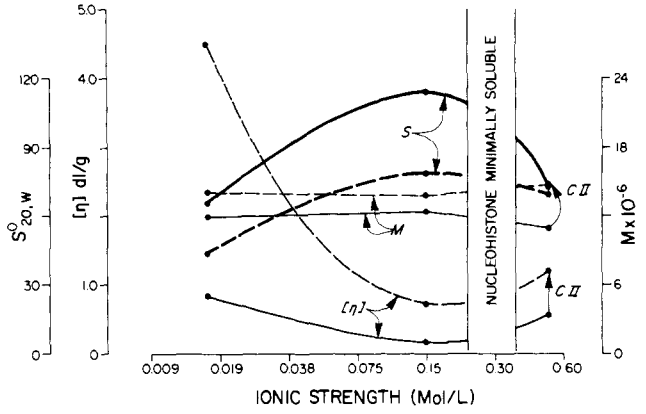

FIGURE 4: Hydrodynamic properties of nucleohistone and F1-depleted nucleohistone derived from it. (—) whole nucleohistone; (- - -) partial nucleohistone. Arrows marked CII indicate the chromatography in 0.5 *M* medium. This is the preparation shown in gels B through E, Figure 1.

centration and gels F and G represent one such lot.

Ionic Strength Effects of F1-Depleted Nucleohistone. Figure 4 presents a plot of sedimentation constant, viscosity, and molecular weight vs. ionic strength for one experiment on both the complete and F1-depleted nucleohistones. The solid lines represent properties of the complete complex while the dashed lines apply to the nucleohistone with F1 removed. On exposure to ionic strengths either higher or lower than 0.15 *M* the complete nucleohistone expands in volume. This general behavior is similar in the F1-depleted nucleohistone.

Figure 4 presents data obtained on a nucleohistone preparation of about 12 million molecular weight. Gels from this preparation are shown in Figure 1B–E. The viscosity of the partial nucleohistone in 0.5 *M* salt was about 1.2 dl/g compared to 0.57 dl/g for the complete nucleohistone in 0.5 *M* and 0.17 dl/g in the original 0.15 *M* buffer. The sevenfold expansion on F1 removal is consistent with the viscosity ratios seen in earlier experiments in 0.6 *M* salt, a condition which would completely dissociate F1. The sedimentation constant is lowered from 116 S in 0.15 *M* to 75 S in 0.5 *M* to 70 S on F1 removal.

The calculated molecular weights are higher after F1 removal than before, across the range of ionic strengths, by 10–25%. A decrease in *M* by 10% is of course expected. We believe the increase in *M* is due to the selective retention of higher *M* partial nucleohistone when trailing fractions of low concentration, from chromatography in 0.5 *M* medium, were discarded.

The partial nucleohistone was dialyzed from 0.5 into 0.15 *M* and a contraction of the molecular domain was indicated by a small rise in *s* and a fall in [η] by 40%. On dialysis into 0.015 *M* a large expansion occurred with a drop in *s* and a rise in [η] by sixfold. The expansion from 0.15 to 0.015 *M* is plotted against $I^{-1/2}$ in Figure 5. In this case the extrapolated [η] is zero at I = 0.4 *M* and $[\eta]_\infty$ is negative. The expansion of the complete nucleohistone in the low *I* medium parallels that already described in Figure 2 and is represented by a line in Figure 3.

Similar experiments on two other preparations, one including viscosity measurements at various *I* down to 0.009 *M*, gave results similar in form to those of Figure 4 (data not shown). However, in those experiments a larger expansion occurred on chromatography in 0.5 *M*, a lesser contraction on dialysis from 0.5 to 0.15 *M*, and a less steep expansion in lower I media. That is, the configuration of those partial nucleohistones was less responsive to changes in *I* than was that of Figure 4.

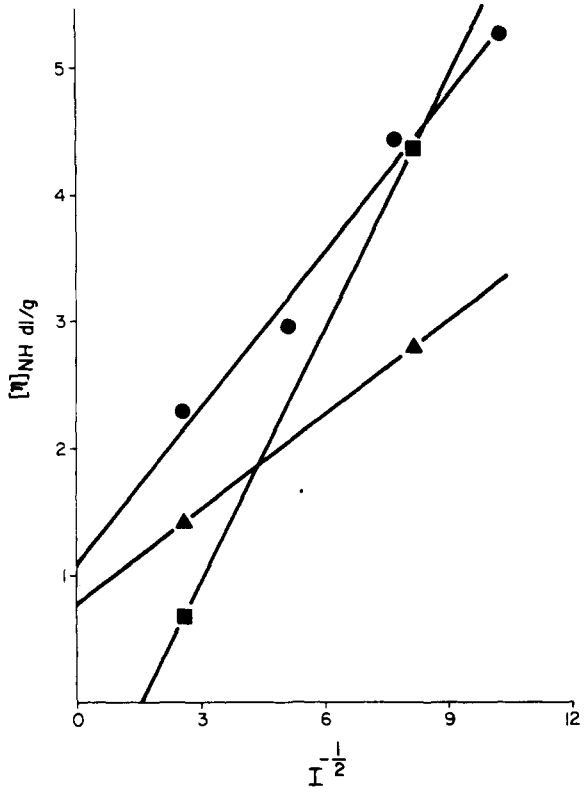

FIGURE 5: Intrinsic viscosity of partial nucleohistone at 0.15 *M* and lower ionic strengths, plotted against the reciprocal square root of ionic strength. (■) The preparation of Figure 4 and gel E, Figure 1; (▲) of gel G, Figure 1.

Plotted against $I^{-1/2}$ in Figure 5, [η] of those lots extrapolates to positive values of [η], resembling in this behavior the majority of flexible polyelectrolytes.

Gels were obtained on only one of those preparations. Excessive histone removal was apparent in this lot, gels F and

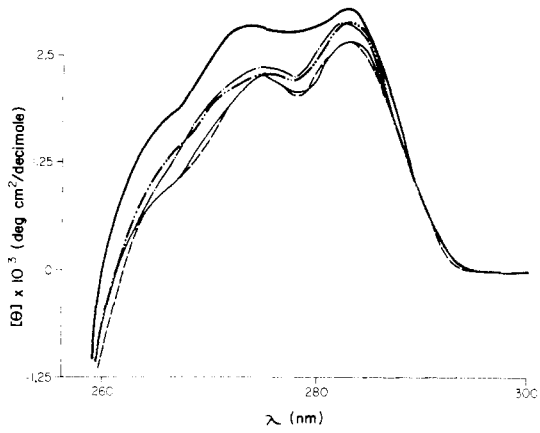

FIGURE 6: Circular dichroism spectra in the 260–300-nm region of soluble nucleohistone and partial nucleohistone. Thin solid line, nucleohistone in 0.15 *M* medium. Heavy solid line, in 0.015 *M*. Dashed line, dialyzed into 0.015 *M* and then back into 0.15 *M*. Dash and dot, dialyzed into 0.6 *M*. Dash and two dots, F1-depleted nucleohistone in 0.15 *M*.

G of Figure 1.

CD Spectrum—Effects of Ionic Strength and of Histone F1 Removal. The complete CD spectrum for the nucleohistone has been previously published (Rees et al., 1974a). Figure 6 shows the 260–300-nm portion of the CD spectrum for the complete nucleohistone in 0.015, 0.15, and 0.6 *M* buffer, the complete nucleohistone which had been taken down to 0.015 *M* and then back to 0.15 *M*, and the partial nucleohistone in 0.15 *M*. The CD spectrum is quite insensitive to changes in ionic strength or even F1 removal. The largest change in the CD spectrum is observed on placing the nucleohistone in 0.015 *M* buffer resulting in a small increase in the positive ellipticity over the range of 260–290 nm. The CD spectrum of F1-depleted nucleohistone is almost identical with that of the complete nucleohistone in 0.6 *M* salt. The maximum ellipticity is increased by less than 10% on removal of F1 with the effects restricted to the 265–275-nm and 280–290-nm regions of the spectrum. The CD spectrum of the nucleohistone exposed to 0.015 *M* ionic strength followed by dialysis back to 0.15 *M* buffer was identical with the spectrum of the original nucleohistone pool. Two points to be made from these data are: (1) histone F1 is not involved in disruption of the DNA base stacking since its removal does not significantly change the CD spectrum toward that of DNA; and (2) CD spectroscopy is a less sensitive probe of the nucleohistone compact state than viscometry as ionic strength changes producing up to tenfold increases in intrinsic viscosity resulted in only small changes in the CD spectrum.

Effects of Pelleting and Shearing on Nucleohistone Solubilization and on Solubility. In order to study the effects of pelleting and mechanical shear on the solubilization reaction, suspensions of the lysed nuclei were either pelleted and resuspended in their original supernatants with a Sorvall Omnimixer, or sheared but not pelleted, or gently swirled, or left unagitated prior to incubation in the presence of Mg^{2+} ion. The rate of solubilization by the endogenous nuclease was markedly reduced in the case of nuclear contents that had been pelleted and then sheared, but largely unaffected by shearing only, prior to incubation. The physical properties of nucleohistone intentionally sheared previous to enzymatic cleavage have not been studied, but an effect of quite gentle shear has become apparent. Our recent practice following the suggestion of Dr. Robert C. Krueger has been to minimize all swirling of the reaction mixtures after nuclei are mixed with buffer (at which time they lyse). With this precaution we obtain more consistent solubilization kinetics and consistently lower viscosity, faster sedimenting nucleohistone. The variability in viscosity mentioned in our previous report (Rees et al., 1974a) we now ascribe to shear damage in the reaction mixture.

Pelleting of the solubilized nucleohistone renders it insoluble. No more than 5% of the pelleted nucleohistone can be resuspended in the original (0.15 *M*) buffer by any degree of agitation we have tried.

Dialysis into or through ionic strengths near 0.3 *M* causes aggregation marked by turbidity and low recoveries in the supernatant on clarification. The aggregates redissolved, however, in either higher or lower ionic strength with recoveries of 90% or better provided the dialyzing nucleohistone was not agitated. Even very gentle agitation of the nucleohistone, in the vicinity of 0.3 *M*, caused poor recovery after dialysis into either 0.15 or 0.6 *M*.

As already reported low-speed shearing in a Potter-Elvejem homogenizer precipitates about 80% of the nucleohistone and the precipitate cannot be redissolved even at higher speeds. High-speed shearing, however, is less effective at precipitating the soluble nucleohistone. The shear in loading ultracentrifuge cells through an 18-gauge needle had no effect on the *s* distribution.

Discussion

The most striking feature of our results is the extrapolation of the intrinsic viscosity of this nucleohistone, measured at 0.15 *M* and lower ionic strengths and plotted against the reciprocal square root of ionic strength, to zero viscosity at a finite ionic strength. Previous workers have been restricted to much lower ionic strengths, by the insolubility of sheared nucleohistone in more concentrated media. In very low salt media, a polyelectrolyte solute may provide much or most of the ionic strength (Pals and Hermans, 1952) and reduced viscosities at one polyion concentration may be misleading. The data of Frisman et al. (1974) are the only measurements on nucleohistone known to us in which intrinsic viscosities were determined over a sufficiently wide range of ionic strengths to permit reliable extrapolation to infinite *I*. Their results when plotted against $I^{-1/2}$ are linear and extrapolate to a positive value of $[\eta]_\infty$. We suggest their results reflect damage from pelleting and shearing.

Our results, shown in Figure 3, contrast markedly with the behavior of DNA (Ross and Scruggs, 1968; Reinert and Geller, 1968; Rosenberg and Studier, 1969), with that of a variety of other polyelectrolytes (Pals and Hermans, 1952; Smidsrod and Haug, 1971, and references therein), and with histone F2a1 (Ziccardi and Schumaker, 1972). The intrinsic viscosity of each of these flexible polyions extrapolates to a positive viscosity at infinite ionic strength, with a linear dependence on $I^{-1/2}$. This common behavior occurs despite aggregation and precipitation at higher *I* (Pals and Hermans, 1952; Smidsrod, 1970). It is predicted by theory (Hermans and Overbeek, 1948; Rice and Nagasawa, 1961; Fixman, 1964). It is intuitively plausible, as it is consistent with a substantial molecular hydrodynamic volume at infinite ionic strength, where the flexible molecule is unperturbed by electrostatic forces, and with a continuous expansion as repulsive forces increase. The volume at infinite *I* represents the most probable distribution of configurations

accessible within the constraints of polymer geometry and excluded volume, which will include solvation effects (Flory, 1953). The expansion results from the equilibrium of electrostatic forces and configurational entropy, as the distribution is biased in favor of more extended configurations (Rice and Nagasawa, 1961).

The unusual behavior of this nucleohistone shows that at high ionic strength, if dissociation of histones did not intervene, it would collapse to a very small volume, and, further, that it would reach this limiting volume at a finite ionic strength, in the range of 0.4–1 *M*. The limiting volume of course cannot be less than that of the unhydrated nucleohistone, and an intrinsic viscosity less than 0.035 dl/g, typical of globular proteins, is most unlikely. The lowest viscosity in Figure 3 is 0.17 dl/g, five times that limit. The actual lower limit to observable viscosities will be set either by steric factors or by the onset of aggregation.

That the limiting volume is approached at finite ionic strength we can only interpret to signify that the ionic strength dependent expansion is resisted by contracting forces which remain positive and finite as the volume approaches zero. Configurational entropy must go through a maximum in some more extended state and cannot provide such a contracting force.

One possibility is that the flexibility of nucleohistone may be elastic, that is, the expansion can occur only by deforming bonds, however slightly, storing energy as elastic stresses, and that some peculiar geometry of the nucleohistone prevents these stresses from going to zero in any configuration accessible to it. Models in which DNA helices are severely twisted or repeatedly bent back, in absence of electrostatic repulsions, could be devised that would exhibit this property.

Alternatively the contracting forces may be intramolecular chemical bonds which are reversible and are not ionic strength dependent. Hydrophobic bonds between proteins spaced some distance apart along the contour length might cause this behavior. If so, it appears that these bonds are not all broken abruptly as ionic strength is reduced, but separate in a progressive, perhaps statistical manner. The chemical forces responsible for the condensation of this nucleohistone must be intramolecular. Intermolecular aggregation at high I which is apparent in the precipitation at 0.3 *M*, is essentially irrelevant and indeed it is probable that many or most of the polyelectrolytes which extrapolate to positive $[\eta]_\infty$ can be salted out. No ionic strength dependent change in molecular weight was seen at 0.15 *M* and lower, the range of the viscosity measurements. Further, both s and n_{sp}/c were found to be independent of nucleohistone concentration, at both 0.15 and 0.009 *M* ionic strength (Rees, et al., 1974a, and data not shown).

The structure responsible for the low viscosity configuration is easily damaged. We observe consistently lower viscosities in 0.15 *M* buffer, for comparable molecular weights, when agitation of the incubation mixture is minimized. The viscosities of this unsheared nucleohistone are much lower than those reported for sheared nucleohistone even when allowance is made (by interpolation or extrapolation on Figure 3) for the low I at which sheared nucleohistone is usually examined (Zubay and Doty, 1959; Bayley et al., 1962; Henson and Walker, 1970; Simpson, 1972; Bartley and Chalkley, 1973). The viscosity of sheared nucleohistone extrapolates to a positive $[\eta]_\infty$ (Frisman et al., 1974). The negative $[\eta]_\infty$ observed consistently with the present nucleohistone becomes variable after partial dehistonization in 0.5 *M* salt.

Whatever the origin of the contracting force it is apparent that the nucleohistone molecule is highly flexible, able to accommodate a tenfold range of viscosity expansion with complete reversibility. This excludes models of nucleohistone structure which are inherently rigid, such as those which postulate histone cross-links between adjacent turns of a superhelix.

A second unexpected result is that the CD spectrum is very insensitive to this reversible expansion. The positive peak at 280 nm, characteristic of the B configuration of DNA, is reduced in this nucleohistone to less than a third of its normal ellipticity and is essentially unaffected by this expansion. This depression of the CD spectrum must mark a gross distortion of the base-stacking geometry of a substantial fraction, at least, of the nucleohistone DNA. Presumably this DNA is severely bent. Its bending is not reversed, as measured by CD, on expansion by tenfold, measured by viscosity.

We infer therefore that two types of bending should be distinguished, one giving rise to a pronounced CD effect, the other participating in the reversible expansion, most probably involving separate regions of the DNA.

Higher ellipticities at 280 nm are observed for sheared nucleohistone (Henson and Walker, 1970; Shih and Fasman, 1970; Simpson and Sober, 1970) while a lower ellipticity has been observed on nuclease-resistant digestion products (Johnson et al., 1972; Sahasrabuddhe and Van Holde, 1974). These observations support the idea that the bending responsible for the CD effect is localized in a part of the DNA and is susceptible to damage by shear.

The removal of histone F1 has been previously shown to have little effect on the CD spectrum of nucleohistone (Bartley and Chalkley, 1973; Vandergrift et al., 1974; Li et al., 1975). Its dissociation in 0.6 *M* ionic strength causes expansion eightfold by viscosity, with negligible CD effect. An expansion by about 100-fold would be needed to reach the configuration of DNA. On reassociation by dialysis into 0.15 *M* the original s and $[\eta]$ are largely recovered. We suggest the incompleteness of recovery is due to some degree of rearrangement of the other histones.

Removal of F1 by chromatography in 0.5 *M* leads to an expansion to eight times the original viscosity. A small decrease in viscosity occurs on dialysis to 0.15 *M*. Lower ionic strengths cause an expansion very like that of the whole nucleohistone, but with viscosities greater throughout. In one case, where the F1 removal was quite selective, these viscosities again extrapolated to zero at about 0.4 *M* ionic strength and to a negative $[\eta]_\infty$. Although F1 caused condensation it was not necessary for the contraction toward zero viscosity.

The reversibility of the expansion on dissociation of F1 shows that it readily finds effective binding sites. The other histones, which are largely dissociated in 1.2 *M* ionic strength, are unable to return to effective sites so easily. Our attempts to reassociate these histones led only to insoluble artifacts.

The idea that the insolubility of most conventionally prepared nucleohistone in 0.15 *M* buffers is due to damage by shearing and pelleting is supported by the observations that pelleting and shearing the lysed nuclei makes the nucleohistone refractory to solubilization; that the soluble nucleohistone cannot be redissolved after pelleting and is precipitated by mild shearing; and that the precipitation of this nucleohistone in 0.3 *M* medium becomes irreversible on exposure

to even very mild shearing. These are indications of important structural rearrangements not of cleavage. It is probable that shearing forces are greatly magnified when they act on a gelatinous aggregate.

The possibility cannot be excluded that the physical properties of this nucleohistone reflect in some degree the histone proteolysis which we have not been able to avoid. Some damage will be inherent in any fragmentation process and the properties of native chromatin must be inferred from the convergence of data from different preparations.

Our results show that the histones other than F1 induce a tendency to contract to a very compact structure, and a gross distortion of the CD spectrum of DNA. F1 induces a further condensation without CD effect. The nucleohistone has great flexibility again without CD effect. These data are compatible with the models of chromatin structure recently proposed by Kornberg (1974) and Van Holde et al., (1974), but not with models in which the histones or bending modes are uniformly distributed.

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