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Structural transition in chromatin induced by ions in solution

Hsueh Jei Li, Ali W. Hu, Rose A. Maciewicz, Patricia Cohen, Regina M. Santella and Catherine Chang

Division of Cell and Molecular Biology, State University of New York at Buffalo, Buffalo, NY 14260 and Department of Chemistry, Brooklyn College of City University of New York, Brooklyn, NY 11210. USA

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ABSTRACT

Structural transition in chromatin was measured as a function of counter ions in solution (NaCl or $MgCl_2$) and of histones bound on the DNA. The addition of counter ions to aqueous solutions of chromatin, partially dehistonized chromatin, and DNA caused a drastic reduction in viscosity and a significant increase in sedimentation coefficient. Transitions occurred primarily at about 2×10^{-3} M NaCl and 1×10^{-5} M $MgCl_2$ and are interpreted as a change in structure of chromatin induced by tight binding of cations (Na^+ or Mg^{++}) to DNA, either free or bound by histones, and is an intrinsic property of DNA rather than of the type of histone bound. At a given ionic condition, removal of histone H1 from chromatin had only a minor effect on the hydrodynamic properties of chromatin while removal of other histones caused a drastic change in these properties. An increase in the sedimentation coefficient of DNA was observed also for protamine·DNA complexes wherein the bound protein contains only unordered coil rather than the α-helices found in histones.

INTRODUCTION

Chromatin structure has been a subject of active research in the past decade. In particular, hydrodynamic methods (viscosity and sedimentation) have been used for investigating the gross structure of chromatin in solution (1-6). Van Holde and co-workers (7-9) have used nuclease digestion as a method to isolate chromatin subunits (10-13) and have measured the hydrodynamic dimensions of these subunits.

In chromatin, particularly in histone-bound regions, at least two types of forces, electrostatic and hydrophobic, are expected to play an important role in determining the structure of a histone·DNA complex. Considerations of such forces led to the suggestion that chromatin subunits could exist in multiple states as compact beads, heterogeneous supercoils or some intermediate structures, depending upon solution conditions (e.g., ionic strength, pH, or urea), histone modifications, binding of other molecules (e.g., nonhistone proteins or RNA) or other environmental factors surrounding the chromatin (14). One aspect of the control of the state of the chromatin subunit structure by external

factors, e.g., urea in solution, has been demonstrated by using circular dichroism (CD) spectroscopy (15-18) and electron microscopy (EM) (18-20). As suggested before (14), urea has been shown to destroy both the ordered secondary structure as well as the non-polar interaction in histones, and eventually causes a transition of the chromatin subunit from a more compact state to a more extended and relaxed state without releasing histones from the DNA.

It has been known for sometime that charge neutralization of the phosphates of DNA by solution ions tends to make a DNA molecule more flexible (21-23). Since not all the DNA phosphates are fully neutralized by histones as judged by a sensitive response of the melting temperature of histone-bound DNA to ionic strength (24), it is suggested that additional charge neutralization in a chromatin subunit might cause a structural transition in the chromatin (14). In order to examine the roles played by solution ions on chromatin structure, we have obtained both viscosity and sedimentation data for solutions of chromatin and NaCl-treated partially dehistonized chromatins as a function of ionic strength, especially at low ionic strength. The results indicate that these hydrodynamic properties of chromatin are very sensitive to the ionic strength. Such dependence will be shown to be an intrinsic property of DNA rather than of histones (both the type and the amount of histones) or of other proteins (e.g., protamine) bound on the DNA.

MATERIALS AND METHODS

Unsheared chromatin from calf thymus was prepared according to the method of Marushige and Bonner (25) as modified by Seligy and Miyagi (26) except that 0.1 mM PMSF (phenylmethylsulfonylfluoride) in 2-propanol was added as a protease inhibitor in the buffers used subsequent to the isolation of the nuclei. Isolated chromatin was dialyzed to 0.25 mM EDTA, pH 8.0 and was sheared in a Virtis homogenizer model 45 at speed setting of 20 or 50 at 15 sec intervals for a total shearing time of 75 sec. In some experiments, 0.1 mM PMSF was added immediately after shearing. Sheared chromatin in 0.25 mM EDTA or in 0.25 mM EDTA plus 0.1 mM PMSF was centrifuged in a Sorvall SS-34 rotor at 10,000 rpm for 10 min. The supernatant was used as sheared chromatin.

NaCl-treated partially dehistonized chromatins were prepared by Sepharose 4B column chromatography (1.2 x 85 cm) (27-29). The eluting buffer was 0.25 mM EDTA, pH 8.0, with or without 0.1 mM PMSF, plus an appropriate NaCl concentration. Sheared and NaCl-treated partially

dehistonized chromatins prepared by a similar method without PMSF were used for some viscosity measurements. As to be described later, no significant difference in the viscosity was observed when PMSF was added to the chromatin solution.

The method of using NaCl-treatment of chromatin to obtain partially dehistonized chromatin is suitable. Although histone rearrangement in NaCl solution after formaldehyde fixation has been reported (30), such rearrangement of histones was shown to be caused primarily by reaction of formaldehyde with histones before fixation rather than by NaCl itself (29).

Shearing as a method to isolate soluble chromatin has also been criticized as causing severe damage to chromatin in terms of its thermal denaturation and CD properties (31,32). Nevertheless, under our conditions of shearing, no significant effects have been observed on the CD spectra of chromatin and the thermal denaturation profiles measured by absorption and CD. Only a samll percentage of histones was shown to be released from the chromatin DNA and to rebind to an exogenous DNA which was added to the chromatin solution as a histone acceptor during shearing (33). Therefore, mild shearing as applied in this report does not seem to affect the overall structure of chromatin, especially when we limit ourselves to the discussion of a transition of the hydrodynamic structure in the presence of NaCl or $MgCl_2$ rather than the determination of hydrodynamic dimensions which may require more stringent conditions of sample preparation and measurement. Chromatin and NaCl-treated partially dehistonized chromatins were finally dialyzed against 0.25 mM EDTA, pH 8.0 for hydrodynamic measurement in NaCl solution and against 1.0 mM Tris, pH 8.0 for similar measurements in $MgCl_2$ solution.

Calf thymus DNA was prepared from the chromatin by pronase treatment or by using the procedure of Marmur (1961).

Protamine was purchased from Sigma Chemical Company. It was dissolved in EDTA buffer and complexed with DNA (1.5×10^{-4} M) by direct mixing. Precipitation curves indicated a non-cooperative binding under this condition as reported before (34). The complexes were then concentrated and dialyzed against EDTA buffer for sedimentation experiments.

Viscosity data were obtained with a Beckman rotating cylinder viscometer as described by Zimm and Crothers (35). The temperature was controlled at 23°C by a constant temperature circulator. The intrinsic viscosity, $[\eta] = \lim_{C \to 0} [\frac{\eta_{rel}^{-1}}{C}]$, is reported, where C is the chromatin

concentration in mole of nucleotide per liter (M) and η_{rel} the relative viscosity.

Sedimentation coefficients of the samples were determined from sedimentation velocity experiments using a Spinco model E analytical ultracentrifuge with Schlieren optics. The samples were centrifuged at 42,040 rpm at 8.5°C; the sedimentation coefficients measured at this temperature were corrected and the corresponding valuse of S_{20} at 20°C are reported.

RESULTS

Intrinsic Viscosity of Sheared Chromatin and Partially Dehistonized Chromatin as A Function of Ionic Strength

The dependence of the viscosity of a polymer on its concentration can be expressed in terms of the following relation.

$$\frac{\eta_{sp}}{C} = \frac{\eta_{rel}^{-1}}{C} = [\eta] + k\,[\eta]^2 C \qquad (1)$$

where η_{rel}, η_{sp}, and $[\eta]$ are, the relative, specific and intrinsic viscosity, respectively, of the polymer, C is the concentration and k is a constant (36).

Plots of eq. (1) for sheared chromatin at three different ionic strengths are shown in Fig. 1. The intrinsic viscosity $[\eta]$, determined from the intercept at zero concentration, and the slope were reduced when the ionic strength of the chromatin solution was increased. Since the main objective in these studies was to examine both the effect of ionic strength and histone dissociation on the viscosity of chromatin, the ratio Y of $[\eta]$ of each sample normalized against $[\eta]$ of chromatin in EDTA buffer is reported. Previously this ratio procedure was used for investigating intercalation of actinomycin in DNA (37).

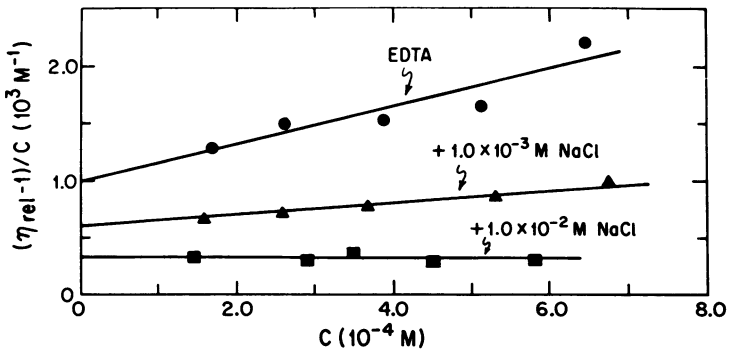

Fig. 1. The viscosity of chromatin at various NaCl concentration. The unit of $[\eta]$ in M^{-1} can be converted to dl/g by dividing the present values by 30.8.

As shown in Fig. 2, the viscosity ratio, Y, of chromatin decreased from 1.0 in EDTA buffer to 0.25 in EDTA buffer + 0.01 M NaCl. The viscosity decreased primarily between 0 and 2 x 10^{-3} M NaCl. For 0.6 M NaCl-treated chromatin which lacks histone H1 (I or F1) (38), a transition pattern similar to that of chromatin, except a slightly higher intrinsic viscosity was obtained. For each of 1.6 M NaCl-treated chromatin and DNA, a transition also occurred but between 0 and 4 x 10^{-3} M NaCl, which was not much greater than the range of NaCl concentration needed for inducing structural transitions in chromatin and 0.6 M NaCl-treated chromatin, i.e., 0 - 2 x 10^{-3} M NaCl. Our results for DNA confirmed the earlier observations

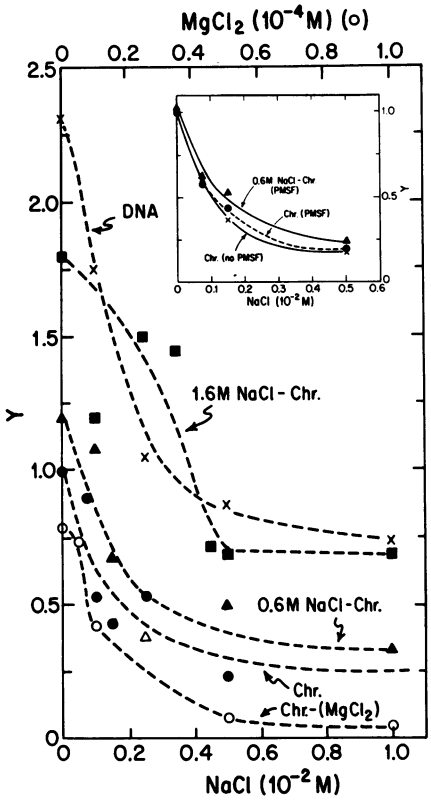

Fig. 2. Ionic strength dependence of intrinsic viscosity of chromatin, partially dehistonized chromatin and DNA. Y is the ratio of intrinsic viscosity of a sample to that of chromatin in EDTA buffer. Chromatin (chr.) and partially dehistonized chromatin, either 0.6 M NaCl or 1.6 M NaCl-treated chromatin (0.6 M NaCl-Chr. or 1.6 M NaCl-Chr.) are indicated. Also inserted in the figure are viscosity ratios of chromatin and 0.6 M NaCl-treated chromatin in the presence, or absence of 0.1 mM PMSF at various NaCl concentrations.

of a strong dependence of intrinsic viscosity of DNA on ionic strength (21).

The above results seem to indicate that the transition of the intrinsic viscosity of DNA induced by NaCl is not sensitive to the binding of various kinds and different amounts of histones to the DNA. Since NaCl in solution exists as ions, Na^+ and Cl^-, and since it is Na^+ but not Cl^- which is bound directly to DNA, it is concluded it is the Na^+ ions which play the dominate role in changing the hydrodynamic structure of DNA and DNA·histone complexes. If this is the case, it is expected that Mg^{++} should be much more effective than Na^+ in inducing a structural transition, since Mg^{++} binds DNA much better than Na^+ (23,39,40). In order to test this hypothesis, the intrinsic viscosity of chromatin as a function of $MgCl_2$ was measured. The results shown in Fig. 2 show a drastic decrease of the viscosity at 1×10^{-5} M $MgCl_2$, a concentration about 100-fold lower than that of NaCl (about 10^{-3} M) needed to induce a similar change in viscosity. Thus, the above hypothesis is supported. The results also support the notion that anions in solution do not seem to play an active role in mediating chromatin structure although they have been shown to play a more important role than the solution cations in inducing conformational changes in free histones (41-44).

Under the same ionic condition, removal of histones from chromatin resulted in an increase in viscosity. For instance, in EDTA buffer, the viscosity ratio, Y, was increased from 1.0 for chromatin to 2.3 for DNA. Removal of histone H1 by 0.6 M NaCl caused only a small increase in viscosity of chromatin which confirms the earlier report of Smart and Bonner (6). The major increase occurred as histones were partially removed by 1.6 M NaCl. Based upon thermal denaturation analysis, 15-20% of DNA base pairs were still complexed by histones in 1.6 M NaCl-treated chromatin, while this value was 40-50% for chromatin treated by 1.0 M, and 50-60% for that treated by 0.6 M NaCl. Thus, the viscosity results summarized in Table I suggest either that those histones removed between 1.0 and 1.6 M NaCl play a more important role in maintaining the hydrodynamic properties of chromatin than do the other histones, or that the reduction of histone coverage on DNA from 40-50 to 15-20% affects the viscosity of chromatin to a greater extent than does the reduction of histone coverage on DNA from about 80% to 40-50%. The former implies structural roles played by specific histones (presumably the slightly lysine-rich histones H2A and H2B) while the latter does not.

Although the presence of 0.01 M NaCl in solution caused a general

reduction of the viscosity of chromatin, partially dehistonized chromatin, and DNA, the effect of histone removal on the viscosity of chromatin and partially dehistonized chromatins measured in 0.01 M NaCl was the same as that measured in EDTA buffer (Table I).

Since a protease inhibitor was not added to those chromatin and NaCl-treated partially dehistonized chromatins used for the experiments of Fig. 2, separate experiments on sheared chromatin in the presence of 0.1 mM PMSF as a protease inhibitor were made in order to exclude the possibility of histone degradation. The results shown in Fig. 2 (insert) for chromatin and 0.6 M NaCl-treated chromatin were identical to those without using PMSF. Electrophoresis of histones, following the procedure of Panyim and Chalkley (45), also showed the presence of all histone species isolated from chromatin either in the presence or the absence of PMSF.

Sedimentation Properties of Chromatin and Partially Dehistonized Chromatin as A Function of Ionic Strength

The concentration dependence of the sedimentation coefficient of a macromolecule can be described by

$$\frac{1}{S} = \frac{1}{S^o}(1 + kC) \qquad (2)$$

where S^o and k are, respectively, the sedimentation coefficient extrapolated to zero concentration of the macromolecule and a constant (36). Such a dependence for chromatin in EDTA buffer plus 0.01 M NaCl is shown in Fig. 3. The sedimentation coefficient extrapolated to zero concentration, S^o_{20}, for the sheared chromatin examined, was 19S in EDTA buffer and 27S in EDTA + 0.01 M NaCl. The addition of 0.01 M NaCl to the chromatin solution resulted in a substantial increase in the sedimentation coefficient.

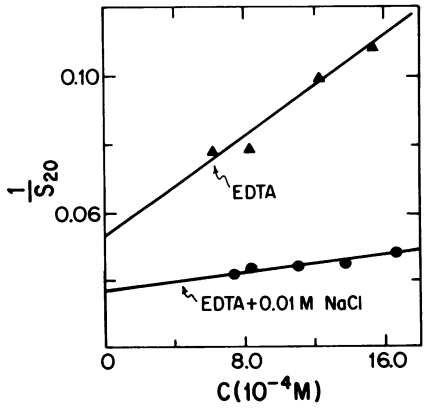

Fig. 3. Concentration dependence of sedimentation coefficient of chromatin.

Because of the concentration dependence of the sedimentation coefficient, the value extrapolated to zero concentration, S^o, should be used for any calculation of molecular weight and dimension of the macromolecule. As to be discussed later, there are reasons to question the validity of using sedimentation data for obtaining these quantities under the present ionic conditions (10^{-3} - 10^{-2} M Na^+, for example). Consequently, our studies were designed only for a qualitative examination of the structural transition in chromatin as a function of both histone binding to DNA and ionic strength in solution by measuring the sedimentation coefficients of chromatin and partially dehistonized chromatins at the same DNA concentration.

For chromatin and 0.6 M NaCl-treated chromatin, the S_{20} was increased from 13S in EDTA buffer to 23-24S in EDTA + 0.01 M NaCl (Fig. 4). For chromatin, partially dehistonized chromatins or DNA, the biggest increase in S_{20} occurred in the range of 1-2 x 10^{-3} M NaCl, similar to the viscosity results shown in Fig. 2. When $MgCl_2$ was used instead of NaCl, an increase in S_{20} of chromatin was observed also as the concentration of $MgCl_2$ was increased from 0 to 1.0 x 10^{-4} M. Parallel to the viscosity results

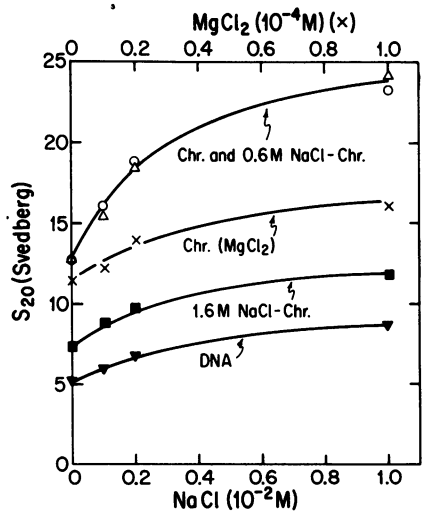

Fig. 4. Ionic strength dependence of sedimentation coefficient of chromatin, partially dehistonized chromatin and DNA. Chromatin in NaCl (O), 0.6 M NaCl-treated chromatin in NaCl (Δ), 1.6 M NaCl-treated chromatin in NaCl (■), DNA in NaCl (▼), all in 0.25 mM EDTA, pH 8.0. Chromatin in $MgCl_2$ and 1 mM Tris, pH 8.0 (X). The concentrations of chromatin and DNA were kept at 8.3 x 10^{-4} mole of nucleotide per liter (A_{260} = 5.4).

shown in Fig. 2, the biggest increase in S_{20} also occurred in 0-2 x 10^{-5} M $MgCl_2$, about 100-fold lower than that of NaCl needed for inducing such a transition. The removal of histone H1 from chromatin by 0.6 M NaCl did not seem to have any detectable effect on the sedimentation coefficient, although thermal denaturation of these two samples did show a much greater fraction of histone-free regions in 0.6 M NaCl-treated chromatin than in the untreated sample. A great decrease in S_{20} occurred when more histones were removed by 1.6 M NaCl. A further decrease in S_{20} occurred when the remaining proteins were removed from the chromatin (Fig. 4).

The ratios of the sedimentation coefficient of NaCl-treated partially dehistonized chromatin to that of chromatin, either in EDTA buffer or in EDTA + 0.01 M NaCl, are given in Table I. At either ionic strength, the removal of histone H1 from chromatin by 0.6 M NaCl did not result in any decrease in sedimentation coefficient. A substantial reduction occurred when more histones (presumably slightly lysine-rich histones) were removed by 1.6 M NaCl. It is noted that the decrease in sedimentation coefficients parallel to that of the increase in intrinsic viscosity. This may not be surprising because viscosity and sedimentation are closely related to each other.

Dependence of Sedimentation Coefficient of DNA·Protamine Complex on the Coverage

The previous hydrodynamic results for DNA (Fig. 1-4) show a strong dependence on both histone binding and counter ions in the medium. In order to see whether or not such a dependence is a unique property for histone·DNA complexes in chromatin, protamine·DNA complexes were prepared by direct

Table I. Effect of Histone Dissociation on Viscosity and Sedimentation Properties of Chromatin

Chromatin treated by NaCl (M)	Intrinsic Viscosity (Y)[a]		Sedimentation (Z)[b]	
	EDTA	EDTA + 0.01 M NaCl	EDTA	EDTA + 0.01 M NaCl
0	1.0 (10^3 M^{-1})	1.0 (0.32 x 10^3 M^{-1})	1.0 (12.8S)	1.0 (23.3S)
0.6	1.2	1.0	1.0	1.0
1.0	1.2	1.1	-	-
1.6	1.8	2.2	0.58	0.51
DNA	2.3	2.3	0.39	0.37

a. Y is the ratio of $[\eta]$ of partially dehistonized chromatin and DNA to $[\eta]$ of chromatin. The value give in ( ) is the measured $[\eta]$ of chromatin. The unit can be converted to dl/g by dividing the value of 30.8. For instance, in EDTA buffer, $[\eta] = 10^3\ M^{-1} = 32.8$ dl/g for chromatin.

b. Z is the ratio of S_{20} of partially dehistonized chromatin and DNA to S_{20} of chromatin. The value given in ( ) is the measured S_{20} of chromatin.

mixing in EDTA buffer. Protamine contains 67 mole percent arginine residues. The complexes prepared by direct mixing showed non-cooperative binding of protamine on DNA and very little ordered secondary structure (α-helix or α-sheet) for bound protamine (34). No globular structure like that of histones in chromatin subunits would be expected for protamine in protamine·DNA complexes. Therefore the latter would be a good system to test whether or not the globular structure of histones in chromatin is responsible for a greater sedimentation coefficient for chromatin than for DNA.

Fig. 5 shows the dependence of S_{20} of protamine·DNA complexes on the input ratio of protamine to DNA, r, reported in amino-acid residue/nucleotide, either in EDTA buffer or EDTA plus 0.01 M NaCl. At either ionic strength, there was a substantial increase in sedimentation coefficient. For instance, from r = 0 to r = 1.0, S_{20} in EDTA buffer was increased from 6 to 11S. According to thermal denaturation results, 70% of DNA base pairs in the complex of r = 1.0 were bound by protamine, which was close to about 80% of base pairs bound by histones in chromatin. Clearly the increase of sedimentation coefficient of DNA complexed by proteins is not a unique property for histones such as in chromatin. Instead it is a general phenomenon of protein binding to DNA.

The above dependence of S_{20} of a protein·DNA complex on its r value also occurred in EDTA + 0.01 M NaCl. This observation is similar to that given in Table I for histone·DNA complexes in chromatin and partially dehistonized chromatins.

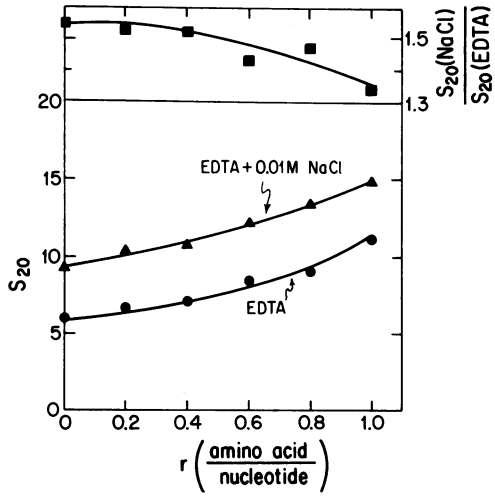

Fig. 5. Dependence of sedimentation of protamine·DNA complexes on the input ratio, r, of protamine to DNA. A_{260} = 5.4 for each complex.

DISCUSSION

Hydrodynamic Properties of Protein·DNA Complexes and Chromatin

For a neutralized macromolecule, its sedimentation coefficient s is related to its frictional coefficient, f, by the following equation

$$s = \frac{M(1-\bar{v}_2\rho)}{Nf} \qquad (3)$$

where N is Avogadro's number, M the molecular weight of the polymer, $\bar{v}_2$ the partial specific volume of the solute and ρ the density of the solution (36). DNA or a protein·DNA complex (chromatin, for example) is certainly not a neutral macromolecule. It does not seem to be warranted to use eq. (3) for a quantitative calculation of the molecular weight of a DNA or a protein·DNA complex without considering the electrostatic field generated by the macroions. Nevertheless, this equation and other viscosity equations derived for neutralized macromolecules could still be useful for a qualitative discussion of the changes in the shape and the dimensions of these charged macromolecules.

Both the decrease in viscosity (Fig. 2) and the increase in sedimentation coefficient (Fig. 4) as the concentration of counter ions in solution is slightly increased (10^{-3} - 10^{-2} M NaCl or 10^{-5} - 10^{-4} M $MgCl_2$) are consistent with a decrease in frictional coefficient f of eq. (3) and the dimension of the equivalent hydrodynamic sphere of chromatin and DNA. Physically a decrease in frictional coefficient represents a change of molecular shape, and can be analyzed as a reduction in the axial ratio of an ellipsoid or as a reduction of the dimension of the equivalent hydrodynamic sphere (See p. 344 of ref. 36).

As the ionic strength in the medium is raised, Rosenberg and Studier (21a) showed a decrease in viscosity and an increase in sedimentation coefficient of DNA. Rinehart and Hearst (22) also observed an increase in the sedimentation coefficient of DNA at higher ionic strengths. The latter authors attributed their observations to a decrease in the frictional factor of DNA which results from charge neutralization of the polyelectrolyte, a reduction of the stiffness of the backbone and a change of long range interactions, such as excluded volume. A conformational change in DNA, from B to C form, for example, was also considered as another possibility. Since the CD of DNA or chromatin is not changed as 10^{-3} - 10^{-2} M NaCl is added to the EDTA buffer (unpublished results), the observed decrease in viscosity and increase in sedimentation coefficient in Figs. 2 and 4 would not originate from conformational changes in DNA.

DNA is a polyelectrolyte. Counter ions in solution tend to condense on the polyelectrolyte (46). The molecules of histones or protamine which are tightly bound on certain segments on DNA could possibly replace the condensed counter ions in these segments. The observation that the dependence of hydrodynamic properties of nucleoprotein complexes (histone·DNA or protamine·DNA) with varied r values on solution ionic strength (Table I and Fig. 5) seems to suggest that the net effect of counter ions on the hydrodynamic dimension of DNA molecules is insensitive to the replacement of some condensed counter ions on DNA by the bound basic proteins.

Role of Histones on Chromatin Structure

Experimental results on chromatin and H1-depleted chromatin (14,24,47) as well as on nuclease-treated chromatin (48-50) indicate that, in histone-bound regions, an octamer of histones H2A, H2B, H3 and H4 bind about 140 base pairs of DNA and form a chromatin subunit while one histone H1 binds 30 - 50 base pairs of a spacer between two neighboring subunits. Conformational distortion in the DNA is greater within the subunits than in the H1-bound spacers since the removal of histone H1 from chromatin has a much smaller effect on the circular dichroism spectrum of DNA than does the other histone (51-54). Hydrodynamic results (both viscosity and sedimentation) also suggest that the removal of histone H1 from chromatin has at most minor effects on the hydrodynamic structure of chromatin, while removal of other histones seems to have a greater effect on the structure measured by viscosity or sedimentation.

The above discussion depicts the DNA structure within histone-bound regions in chromatin as composed of more distorted regions (about 140 base pairs) separated by extended regions (about 40 base pairs). Although, from both CD and hydrodynamic results, these differences seem to be attributable to the binding of different types of histone to these two sets of regions in DNA, one important and relevant question to be presented below has not been seriously considered.

Previously it was proposed that larger histone subunits including the octamer and H1 are formed prior to cooperative binding of these subunits to DNA (14). This hypothesis was presented in order to explain the regular distribution of histones (octamer - H1-octamer -H1....) and DNA structure (condensed-extended-condensed-extended....) along a chromatin molecule. This hypothesis implies that it is histone H1 which prevents the DNA in spacer regions from being bound by the octamer as well as from being distorted or condensed. In other words, this hypothesis implies that histone

H1 plays an active role in determining chromatin structure. However, using the analogy of dye binding to DNA in which intercalation of a dye to one site automatically excludes the adjacent sites from being intercalated due to structural restrictions set by the DNA (23,55,56), it might be possible that the binding of an octamer to one site (140 base pairs) will cause structural distortion in this region which will automatically exclude the adjacent regions (X base pairs where X could be about 50) on both sides from being severely distorted. Such exclusion could possibly dictate that these regions cannot be bound by the octamers but be free or bound by other proteins such as histone H1 or nonhistone proteins whose binding to DNA could distort the structure of the latter only slightly or not at all. This new hypothesis suggests that the generation of spacers is not determined by histone H1 but by the octamer and DNA. To distinguish the above two hypotheses, i.e., whether histone H1 plays an active or a passive role in generating spacers with extended structure in chromatin, one can probably examine simultaneously the length of spacers and their conformation in chromatin or reconstituted chromatin with or without the presence of histone H1.

Transition of the Gross Structure of Chromatin

Recently Lewis et al. (57) reported an increase in sedimentation coefficient and a decrease in intrinsic viscosity of 0.15 M NaCl-soluble chromatin when the ionic strength was increased from 0.009 to 0.15 which covered a salt range much greater than that reported here (10^{-3} - 10^{-2} M NaCl). Finch and Klug (58) also reported the existence of a more condensed structure of chromatin in 0.2 mM Mg^{++} than in 0.2 mM EDTA under the electron microscope which is in agreement with a decrease in viscosity and an increase in sedimentation coefficient when the $MgCl_2$ concentration was raised from 10^{-5} to 10^{-4} M (Figs. 2 and 4). Both reports as well as the present one all indicate a strong dependence of chromatin structure on ionic strength. These observations could possibly measure the type of structural transition in chromatin induced by ionic strength as suggested earlier (14). However, as discussed above, such transitions are determined primarily by the binding of cations to the DNA, both in histone-free and -bound regions, rather than by histone-histone interactions.

Based upon our observations and the fact that chromatin becomes very insoluble in approximately 0.1 M NaCl or 3-4 mM $MgCl_2$, the following structural changes are suggested to describe the transition of the dimension and gross structure of chromatin molecules as the ionic strength in

solution is increased.

$$\text{Extended Chromatin} \xrightarrow[\text{or } 10^{-5} \text{ M } MgCl_2]{10^{-3} \text{ M NaCl}} \text{Flexibly Coiled Chromatin} \xrightarrow[\text{or } 10^{-3} \text{ M } MgCl_2]{10^{-1} \text{ M NaCl}}$$

$$\text{Aggregated Chromatin} \xrightarrow[\text{or } 1.0 \text{ M } MgCl_2]{3.0 \text{ M NaCl}} \text{DNA + Histones}$$

At an extremely low ionic strength, due to strong electrostatic repulsion, chromatin probably could exist in an extended form. By increasing the ionic strength to 10^{-3} M NaCl or 10^{-5} M $MgCl_2$, for example, charge neutralization and other effects could make the molecule more flexible and coiled. By increasing the ionic strength another 100-fold, e.g., about 0.1 M NaCl or 3-4 x 10^{-3} M $MgCl_2$, chromatin becomes aggregated and can be pelleted by low speed centrifugation. Such an induction of aggregation could be a result of further charge neutralization on the chromatin molecule, such as that on residual charges on DNA and histones and dehydration in the chromatin molecule. Indeed, Griffith (59) showed that the SV 40 mini-chromosomes appeared as a string of beads in the electron micrograph at 0.015 M NaCl and as packed beads at 0.15 M NaCl. The diameter of chromatin fiber was reported to be reduced from 200 Å in 1mM Mg^{++} from the chromatin (60). If the ionic strength in the medium is increased further, 0.4 - 3.0 M NaCl (5,24,38) or 0.1 - 1.0 M $MgCl_2$ (61,62) histones are dissociated from DNA.

It is interesting to note that the concentration required for $MgCl_2$ in the first two transtitions of macroscopic parameters of the structure of chromatin is about two orders of magnitude lower than that required for NaCl. On the other hand, the $MgCl_2$ concentration for dissociating histones from DNA is only a factor of two to four lower than the corresponding NaCl concentration. Perhaps, structural transtitions are controlled primarily by strong binding of cations (Na^+ or Mg^{++}) to DNA, stronger for Mg^{++} than for Na^+ (39,40), while histone dissociation is controlled by both the binding of anions (Cl^- in both cases) to histones and the binding of cations to the DNA. In agreement with this view is the report that $MgCl_2$ is only slightly more effective than NaCl in inducing conformational changes in histone H4 (42).

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