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Biochemistry

Conformational changes of the chromatin subunit

(ν_1 bodies/ionic strength effects/hydrodynamic models/crosslinking)

V. C. GORDON*, C. M. KNOBLER*, D. E. OLINS†, AND V. N. SCHUMAKER*

* Department of Chemistry and Molecular Biology Institute, University of California, Los Angeles, California 90024; and † University of Tennessee-Oak Ridge, Graduate School of Biomedical Sciences, and Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

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ABSTRACT **Hydrodynamic studies on monomer chromatin subunits (ν_1) as a function of ionic strength (0.7 mM to 100 mM KCl) indicate two salt-dependent conformational transitions. An abrupt transition occurs at about 7.5 mM ionic strength. Decreasing the ionic strength from 10 to 5 mM results in a decrease in $s_{20,w}$ of the ν_1 from 11.1 to 9.9 S. The diffusion coefficient $D_{20,w}$ decreases from 3.3 to 2.7×10^{-7} cm^2 sec^{-1}. The ν_1 crosslinked with formaldehyde at 10 mM ionic strength do not undergo a similar salt-dependent change in $s_{20,w}$. Another transition is observed at about 1 mM ionic strength; $s_{20,w}$ decreases to 9.4 S and $D_{20,w}$ decreases to 2.2×10^{-7} cm^2 sec^{-1}. Throughout the entire salt range the molecular weight of the ν_1 remains reasonably constant, implying that salt-dependent changes in the frictional coefficient are being observed. Various hydrodynamic models are considered as possible interpretations of the observed changes in the frictional coefficient.**

There is now considerable evidence that eukaryotic chromatin consists of a repeating subunit (ν_1) composed of eight "inner histones" (two each of H4, H3, H2A, and H2B) associated with approximately 140 base pairs of DNA (1–3). In native chromatin these subunits are joined into a polymeric array and associated with the H1 class of histones. Large quantities of monomer subunits (ν_1) have been isolated, subfractionated, and characterized, following digestion with micrococcal nuclease (4). In the present study of ν_1, hydrodynamic evidence from quasielastic light scattering and from sedimentation studies indicates the existence of two salt-dependent conformational transitions.

MATERIALS AND METHODS

Monomer Particle Preparation. Monomer particles (ν_1) from chicken erythrocyte chromatin were isolated by zonal ultracentrifugation as described previously (4, 5). The fraction of monomers that is soluble in 0.1 M KCl (4) was utilized in the present studies. Prior to the hydrodynamic experiments, the KCl-soluble fraction of ν_1 was dialyzed extensively against 0.2 mM EDTA (pH 7.0). These particles contain no detectable H1 or H5, and the DNA averages 140 nucleotides, with a narrow distribution.

Both sedimentation and diffusion of ν_1 preparations were measured as functions of ionic strength. A 0.2 mM EDTA buffer (pH 7.0) was used to obtain the solution at an ionic strength of 1 mM. The ionic strength was increased by additions of a concentrated solution of KCl in 0.2 mM EDTA, and was decreased by dilutions with 0.02 mM EDTA (pH 7.0). Densities of all solutions were determined with a Mettler–Paar precision density meter. Samples were filtered with 0.22-μm Nucleopore filters prior to study. The temperature was recorded with a thermistor to 0.1°.

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Sedimentation Studies of the Monomer Subunit. Sedimentation coefficients were obtained on a Beckman model E ultracentrifuge at 48,000 rpm and corrected to 20°. The studies were performed on solutions with A_{260} = 0.4 to 0.6, using a photoelectric scanner to follow the migrating boundaries. Previous studies (4) have shown that the sedimentation coefficient of ν_1 exhibits negligible concentration dependence below A_{260} = 1.0.

Diffusion Studies of the Monomer Subunit. Diffusion coefficients were determined by quasielastic light scattering. A self-beating spectrometer was used to study the light scattered by the monomer particles. The light had a wavelength of 5145 Å. The autocorrelation function was obtained with a Saicor 43 autocorrelator operating in the clipped mode. The autocorrelation function was fitted to a function $y(t) = A + Be^{-t}\tau$ in which τ is $1/2Dk^2$ and the scattering vector k is a function of the refractive index n, the wavelength λ_0, and the scattering angle θ, and is given by $k = (4\pi n/\lambda_0) \sin(\theta/2)$ (6). The correlation function was measured for several ionic strengths at four angles between 45° and 90° and τ^{-1} was plotted versus k^2. The diffusion coefficient was obtained by a linear least squares analysis of the line.

The operation of the spectrometer was tested by a measurement of D for 1090 Å polystyrene latex spheres in aqueous suspension. Detergent was added to prevent aggregation. A monolayer of the detergent bound to the polystyrene would cause an increase in the effective diameter to about 1130 Å (7). For a sphere of this size, the Stokes–Einstein relationship gives $D_{20,w} = 0.38 \times 10^{-7}$ cm^2 sec^{-1} (10^{-7} cm^2 sec^{-1} = 1 Fick unit, F). The experimentally determined value for the polystyrene spheres was 0.378 ± 0.012 F.

The studies were performed on solutions with A_{260} in the range of 18 to 60. No concentration dependence of $D_{20,w}$ was found.

Crosslinking Studies. For some of the studies reported below, monomer particles were crosslinked with 1% or 4% formaldehyde [Beckman–Cole Co., stored as a 37% (vol/vol) solution in water with 15% (vol/vol) methanol as a preservative]. After 15 or 90 min of fixation of the subunits at 4°, the monomer solution was diluted to the desired ionic strength and the sedimentation coefficients were obtained.

RESULTS

The measured sedimentation coefficients corrected for the viscosity and density of the solvent are shown in Fig. 1 as a function of the logarithm of the ionic strength. These data are a composite of four separate studies and three different preparations of monomer particles. Transition 1, in which the sedimentation coefficient increases from 9.4 to 9.9 S, occurs between ionic strengths of 0.7 and 2.0 mM. Transition 2, in which

Abbreviation: F, Fick unit, 10^{-7} cm^2 sec^{-1}.

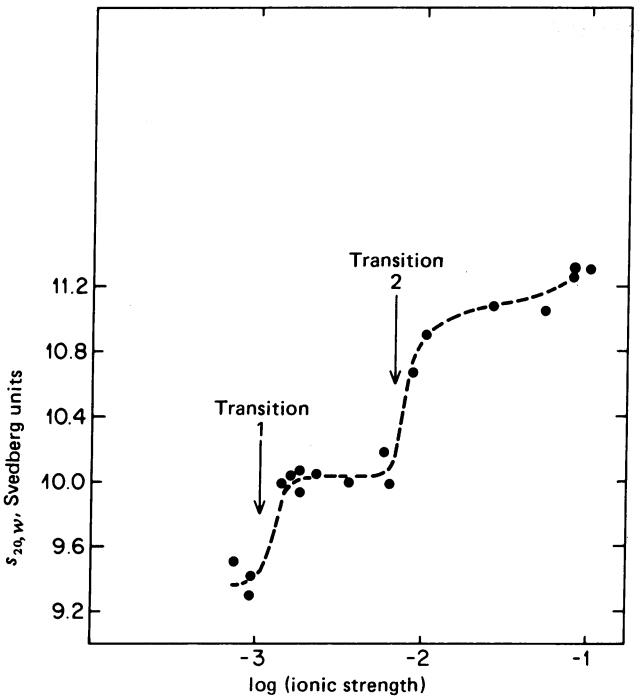

FIG. 1. $s_{20,w}$ plotted as a function of the logarithm of the ionic strength. Initially the monomer particles were prepared in 0.2 mM EDTA (pH = 7.0), at an ionic strength of 0.001 M. Then the ionic strength was increased by additions of 0.25 M KCl in 0.2 mM EDTA. For centrifugation the temperature was regulated close to 20°, and the speed was 48,000 rpm.

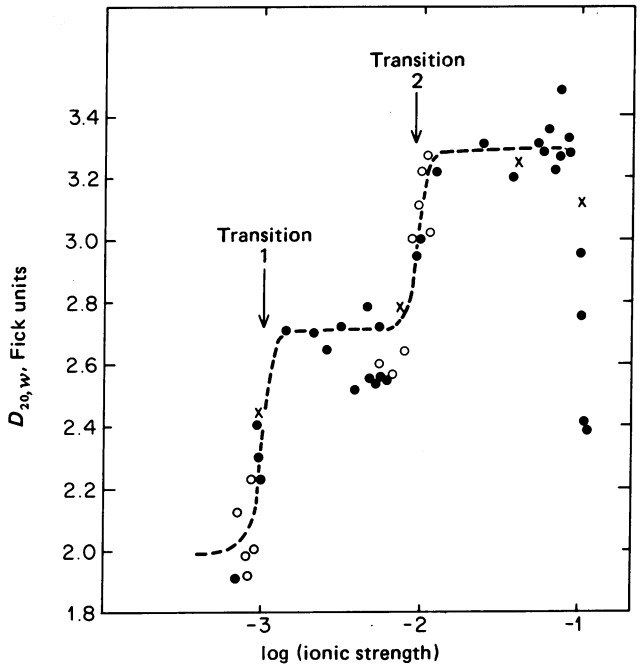

FIG. 2. $D_{20,w}$ plotted as a function of the logarithm of the ionic strength. The monomer particles were prepared in 0.2 mM EDTA (pH = 7.0) at an ionic strength of 0.001 M. The autocorrelation function was measured and fit to a single exponential plus baseline to obtain D. The first study was at four angles (×). The second study was at 90°, with the ionic strength increased by additions of 0.25 M KCl in 0.2 mM EDTA (●). The third and fourth studies investigated the reversibility of transition 1 and transition 2 and data were obtained by dilution (○).

the sedimentation coefficient increases from 9.9 to 10.9 S, occurs between ionic strengths of 5 and 10 mM.

The measured diffusion coefficients corrected for the temperature and viscosity are plotted as a function of the logarithm of the ionic strength in Fig. 2. These data are also a composite of four studies and three different monomer preparations. In the first study correlation times were determined at four different angles and a linear least squares fit for τ^{-1} versus k^2 was performed to obtain D.

A second monomer preparation was studied only at 90°. The particles were initially in 0.02 mM EDTA and then 0.25 M KCl was added to increase the ionic strength. A third study was performed on the second preparation to determine the reversibility of the transition. The KCl solution was added to obtain a final ionic strength of 0.010 M, then 0.2 mM EDTA was added to lower the ionic strength. A fourth study on a third monomer preparation was undertaken to investigate the very low ionic strength transition. These data points were obtained by dilution of the 0.2 mM EDTA with 0.02 mM EDTA (pH 7.0).

Monomer particles were crosslinked with 1% formaldehyde at an ionic strength of 10 mM for 90 min at 4°. When the ionic strength was lowered to 3 mM by dilution, $s_{20,w}$ remained 11.1 S (Table 1). Monomer particles crosslinked for only 15 min at an ionic strength of 10 mM and diluted to 3 mM had an $s_{20,w}$ of 10.5 S. When a control at an ionic strength of 3 mM was crosslinked with 1% formaldehyde for 90 min at 4°, the $s_{20,w}$ was 10.2 S. Monomer particles were also crosslinked with 1% formaldehyde at an ionic strength of 1.5 mM. When the ionic strength was lowered by dilution to 1 mM, the $s_{20,w}$ remained 10.3 S. Control ν_1 treated with formaldehyde at an ionic strength of 1 mM had an $s_{20,w}$ of 9.5 S.

When the monomer particles ($A_{260} \sim 20$) were crosslinked with 4% formaldehyde for 90 min at 4° at an ionic strength of 10 mM and then studied at final ionic strengths of 10, 5, and 1 mM, $s_{20,w}$ remained 11.2–11.3 S. When monomer particles at 0.5 mM with $s_{20,w}$ of 9.4 S were crosslinked with 1% formaldehyde for 90 min at 4°, the $s_{20,w}$ still increased to 11.2 S upon the addition of salt to a final ionic strength of 10 mM.

Molecular weights for ν_i at different ionic strengths were estimated from the measured values of $s_{20,w}$ and $D_{20,w}$. These

Table 1. Crosslinking studies with formaldehyde

Formaldehyde fixation			Ionic strength during sedimentation measurement, mM	$s_{20,w}$, S	Transition investigated
Ionic strength, mM	Time, min	Formaldehyde concentration, %			
10	0	0	10	11.1	2
10	15	1	3	10.5	2
10	90	1	3	11.1	2
3	0	0	3	10.1	2
3	90	1	3	10.2	2
1.5	90	1	1.5	10.3	1
1.5	90	1	1	10.3	1
1	90	1	1	9.4	1
1	0	0	1	9.3	1
1	90	1	10	11.2	1 and 2 (reversibility)
10	90	4	10	11.3	1 (control)
10	90	4	5	11.2	1
10	90	4	1	11.3	1 and 2

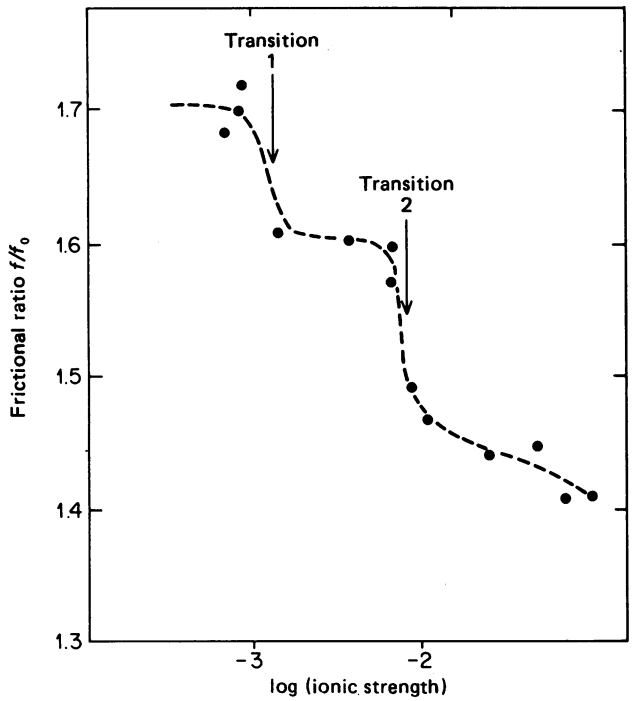

FIG. 3. The frictional ratio, f/f_0, plotted as a function of the logarithm of the ionic strength derived from the sedimentation data.

values were slightly higher than those reported in a previous study (4), but clearly indicate that particle association or dissociation is not occurring during either transition 1 or 2.

Finally, a decrease of D and an increase of intensity of the scattered light was observed at ionic strengths greater than 90 mM. This change is attributable to aggregation because the ratio of s/D increases. The aggregation at these high ionic strengths can also be observed as turbidity between 300 and 500 nm. The aggregates can be removed by filtering with a 0.22-μm Nucleopore filter or by spinning at 7,000–10,000 rpm. This aggregation does not appear to be due to gross compositional differences of the soluble and insoluble subunits, but it does seem to depend on the concentration of particles. These observations are consistent with the work of Sahasrabuddhe and Saunders (8), who studied salt-induced structural changes in nucleosomes. They found that the addition of 20 mM $(NH_4)_2SO_4$ to a 0.01 M Tris/EDTA buffer (pH = 7.8) of human placenta nucleosomes resulted in an increase in turbidity that they attributed to precipitation. Similar results were obtained for the addition of KCl at equivalent ionic strengths.

DISCUSSION

Chick erythrocyte chromatin ν_1 exhibited a transition in $s_{20,w}$ and $D_{20,w}$ with an increase in ionic strength from 5 to 10 mM. The transition was reversible: dilution of ν_1 solutions with 0.2 mM EDTA starting an an ionic strength of 10 mM caused a decrease in $s_{20,w}$ and $D_{20,w}$. The corresponding increase was observed when the ionic strength was started at 5 mM and raised. The quantity RTs/D (R is the gas constant; T, absolute temperature) is equal to the buoyant molecular weight. As one proceeds across transition 2 from 5 to 10 mM, this quantity drops from 91,400 to 84,000, or by 9%. Because it would be expected that the apparent buoyant molecular weight would vary a little with ionic strength at these low salt concentrations, we believe these data indicate that the molecular weight remains essentially constant. Therefore, we feel that this transition is best interpreted as a conformational change. A second reversible transition in $s_{20,w}$ and $D_{20,w}$ occurred between ionic strengths of 0.7 and 2.0 mM. This transition can also be interpreted as a conformational change. We have no information concerning the time dependence of these conformational changes; our measurements were taken about half an hour after adjustment of the ionic strength.

Fig. 3 presents a plot of the frictional ratio, f/f_0, derived from the observed sedimentation data versus the logarithm of the ionic strength. A similar plot is obtained from the diffusion data.

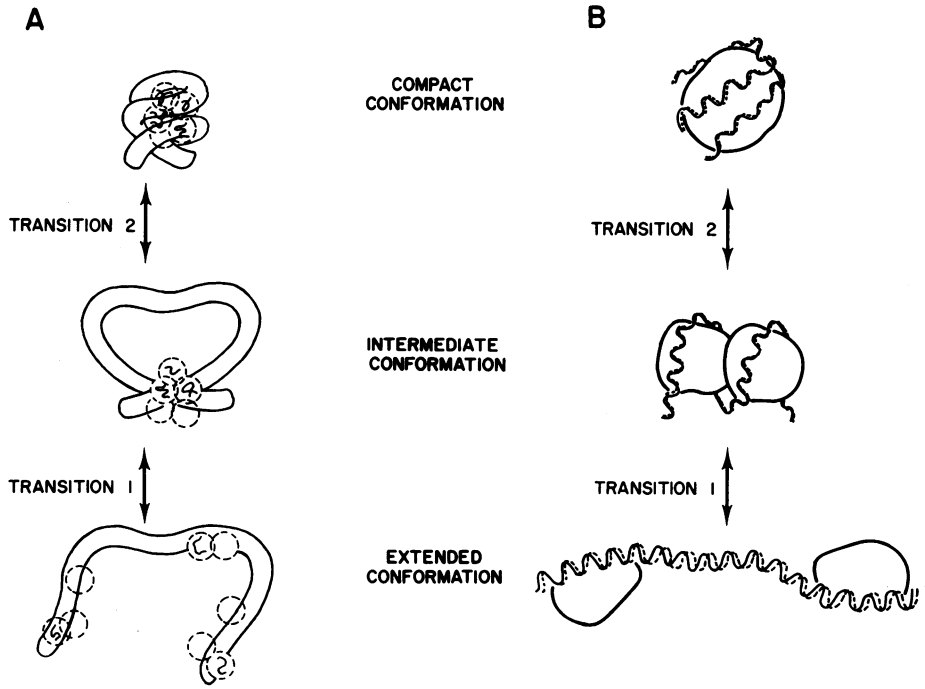

FIG. 4. Representations of possible conformational changes of the monomer subunit (ν_1) based on (*A*) Olins *et al.* (9) and (*B*) Weintraub *et al.* (10).

The abrupt changes in f/f_0 are indicative of changes in the conformation of the ν_1.

Our data are consistent with two recently proposed models for the nucleosome (9, 10). The model of Olins *et al.*, was used to explain studies on the effect of urea on the ν_1. The protein core and the DNA are pictured as responding differentially to small amounts of urea. The ν_1 becomes larger as the outer DNA shell is looped out away from the protein core (Fig. 4*A*) This type of structure is consistent with our measurements for the intermediate conformation if the change in f/f_0 is interpreted as a 14% increase in the effective hydrodynamic radius of the compact conformation due to the unfolding of the DNA. Additional evidence for the possibility of DNA unfolding from the ν_1 is presented by Lilley and Tatchell (11). They investigated the changes in circular dichroism, melting temperatures, sedimentation, and digestion patterns after trypsin digestions. They interpreted changes in $s_{20,w}$ from 11.2 to 10.5 S with mild digestion and a further decrease in $s_{20,w}$ from 10.5 to 9.7 S as structural unfolding of the DNA as the NH_2-termini of the histones were cleaved.

A second model for the ν body has been proposed by Weintraub *et al.*, (10). Their model is based on a protein core composed of two symmetrical heterotypic tetramers. Central to their model is the possibility of unpairing of the two tetramers into "half-nucleosomes." This unpairing can occur by partial loss of the DNA–histone interactions, resulting in an ellipsoid conformation of axial ratio approximately 3–4 based on a hydrated sphere 110–125 Å in diameter in the compact conformation (12). This model is also consistent with our measurements of the change from the compact conformation to the intermediate conformation associated with transition 2. In a prolate ellipsoid model with a semi-axis of rotation a = 130 Å the frictional coefficient would increase by 14%, assuming f/f_0 = 1.0 for the compact sphere 110 Å in diameter. This conformational change is pictured in Fig. 4*B*.

Our measurements are also consistent with a unit particle identified by Langmore and Wooley with the electron microscope (13). By using dark-field electron microscopy, they saw a disc-shaped particle 135 × 50 Å. The particles were deposited on the carbon film in 1 mM $NaPO_4$ at pH 7.0, blotted, and air dried. Many of the particles appeared either U-shaped or circular with a hole in the center. This appearance suggests that the DNA is looped out around the protein core. The dimensions given by Langmore and Wooley are fairly consistent with our measurements for the intermediate conformation if one assumes that the ν_1 changes in asymmetry without changing in volume as the ionic strength is decreased below 10 mM. An oblate ellipsoid of dimensions 160 × 50 Å would have a f/f_0 of 1.13 relative to a hydrodynamically equivalent sphere of diameter 110 Å (12). This is slightly larger than the dimensions determined by Langmore and Wooley.

When the ν_1 in the compact conformation is crosslinked with formaldehyde at ionic strengths above 10 mM, no decrease in $s_{20,w}$ is observed when the ionic strength is lowered to 5 or 1.0 mM by dilution (Table 1). Hydrodynamic properties determined at low ionic strength may be influenced seriously by charge effects. Therefore, the results of the formaldehyde experiments are reassuring evidence that, over the range of ionic strengths employed in thise studies, no alterations in hydrodynamic properties due to charge effects occur for the formaldehyde-treated, presumably crosslinked particles.

When the ν_1 is crosslinked with formaldehyde, both DNA–histone and histone–histone crosslinkages are formed (14, 15); hence our crosslinking studies support the interpretation of transition 2 and transition 1 as a conformational change. However, the studies do not distinguish the two models.

Transition 1 occurs in the ionic strength range 0.7–2.0 mM. Over this range, the quantity RTs/D decreases from 114,800 to 91,400 daltons. Once again we suspect that this drop of 21% in the apparent buoyant molecular weight is an artifact due to the very low ionic strength value at the extreme of the transition. We feel that transition 1 is also best interpreted as a conformational change.

Below 1.5 mM the DNA may become fully extended and the two halves of the histone core separate. Bloomfield *et al.*, developed a hydrodynamic model of two frictional elements on a rod (16), which could represent the unpaired conformation as described by Weintraub *et al.* The ν_1 as pictured by Olins *et al.* (9) at low urea concentrations could undergo a further transition to this extended conformation. A structure in which two half-nucleosomes of radius 40–45 Å have a center-to-center distance of 400 Å would have a 40% larger f/f_0 relative to the compact conformation. Such a change in f/f_0 is consistent with the observed changes in $D_{20,w}$, but is larger than the changes observed in $s_{20,w}$. The transition is pictured in Fig. 4. We have looked for half-nucleosome structures of this type in the electron microscope by using preparations dried on carbon-coated grids from solutions of low ionic strengths, then stained with 5 mM uranyl acetate (9). We do not see them, but it is possible that refolding occurred upon staining. Lawrence (17) reported that half-nucleosomes have been observed by Chambon, who used polyamine-coated grids, which might tend to bind the particles in the extended conformation when they are deposited at low ionic strength.

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