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Salt induced transitions of chromatin core particles studied by tyrosine fluorescence anisotropy

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ABSTRACT

Chromatin core particles containing 146 base pairs of DNA have been found to undergo a single defined transition below 10 mM ionic strength as studied by both sedimentation velocity and tyrosine fluorescence anisotropy. A method is described for the preparation of such core particles from chicken erythrocytes with greater than 50% yield.

INTRODUCTION

Chromatin core particles are particularly stable intermediates in the micrococcal nuclease digestion of eucaryotic chromatin. Generally accepted as a primary structural unit of eucaryotic chromatin, the core particle consists of 146 base pairs of DNA wrapped about an octamer of two each of the inner histones, H2a, H2b, H3 and H4 (1).

With few exceptions (2,3) reports of core particle preparations have not included estimates of the yields obtained. Our early efforts to produce core particles resulted in typical yields of 10-15% of the starting DNA. We therefore developed an approach to improve the yield. The preparation of chicken erythrocyte core particles described here is relatively fast (2-3 days), conservative of the nuclease, and easily scaled to larger or smaller quantities than the 40 mg of core particles typically obtained (50-60% yield). The approach includes an improved method for removal of histones H1 and H5 which does not expose the chromatin to high salt concentrations. High ionic strength has been shown to cause substantial modifications of chromatin structure (4) including the formation of spacerless oligomers of nucleosome cores (4-6). Quantita-

tion of the linker and core DNA indicates that essentially all of the erythrocyte chromatin is in the form of nucleosomes with an,average repeat length of 210 base pairs of DNA.

Chromatin core particles have been reported to undergo two transitions at low ionic strength: one near 1 mM (7-10) and a second near 6 mM (7, 8, 11). We report one distinct transition in this region, detected by sedimentation velocity and by tyrosine fluorescence anisotropy. The transition occurs below 0.5 mM ionic strength and does not appear to be complete even at 0.03 mM. Possible reasons for the differences between our results and those of others are discussed.

MATERIALS AND METHODS

Micrococcal nuclease was obtained from Worthington; it was dissolved at 12,500 units/ml in 0.05 M Tris/HCl, pH 7.5, 20% glycerol and stored at -20°. Pronase from Calbiochem was self-digested at 10 mg/ml in 0.1 M Tris/HCl, 10 mM EDTA, pH 8.0, 0.1 M NaCl for 1 hour at 37° and stored at -20°. Samples for DNA electrophoresis were digested for 3 hr at 37° with 0.4 mg/ml self-digested pronase in 0.13 M NaCl and 1.8% lauroyl sarcosine (Sigma). Details of the electrophoresis methods are included in the respective figure captions.

Glass distilled water was used throughout these experiments and had a conductivity of less than 0.5 μmhos/cm. Chemicals used were of analytical reagent grade.

Sedimentation and fluorescence measurements. Sedimentation was done on a Beckman model E ultracentrifuge operated at 40,000 rpm and 20°.

Fluorescence measurements were made on a computer interfaced fluorescence anisotropy spectrometer (12). Excitation was with the 280 nm line of a mercury-xenon arc isolated through two grating monochrometers and polarized with a double Glan-Taylor prism polarizer. Emission was detected at right angles to both the propagation and polarization directions of the exciting light. Emission at 325 nm was isolated by a grating monochrometer after passage through a double Glan-Taylor prism polarizer oriented either parallel ($F_{//}$) or perpendicular ($F_{\perp}$) to the excitation polarizer. Stray excit-

ing light was removed with a Corning 0-54 cut-off filter (less than 1% transmission below 300 nm). A sensitivity correction (12) was made for differential light transmission in the two polarization modes. After correction, fluorescence intensity was computed as $F = F_{//} + 2F_{\perp}$ and polarization was calculated as $R = [F_{//} - F_{\perp}]/F$ (12). Temperature was maintained at 20°.

In a typical experiment, a core particle stock solution containing 3 mg/ml was dialysed extensively against 1 mM Tris-HCl, 0.05 mM EDTA, pH 7.5. Samples for fluorescence (and sedimentation) measurements were prepared by dilution of the stock solution into water or salt solution to a core particle concentration of 0.1 mg/ml (0.5 μM). The salt solutions were made by dilutions from a 1.00 M stock. The reported ionic strengths were calculated based on the dialysis buffer concentration (assuming Tris-HCl to be fully ionized) and the added salt. However, since the conductivity of the dialysed core particle stock solution was two to three times that of the dialysis buffer, these ionic strengths may be low by as much as 0.07 mM. The high ionic strength of the dialysed core particle solution probably derives from a limiting of the dialysis rate by the polyelectrolyte concentration in the dialysis bag. Core particles are estimated to have an excess negative charge near pH 7 of greater than 100 units per particle (9). Thus a 15 μM (3 mg/ml) solution of core particles requires a monovalent cation concentration of at least 1.5 mM for electrical neutrality.

Core Particle Preparation. Blood was obtained by heart puncture from white leghorn hens and mixed with about 1/5 volume 6% sodium citrate on ice. After filtering through cheesecloth, the erythrocytes were pelleted at 2000xg for 10 min and washed three times with cold 0.15 M NaCl, 0.015 M sodium citrate. The buffy coat was removed and the packed erythrocytes stored at -70°.

Five ml of frozen, packed erythrocytes were lysed at room temperature by thawing while pipetting with 10 ml of 0.01 M Tris/HCl, pH 7.2, 0.15 M NaCl, 0.5 mM phenylmethylsulfonyl fluoride (PMSF)[1], 1% isopropanol. The PMSF in isopropanol was added immediately before use. After dilution to

50 ml the nuclei were pelleted at 2000xg for 10 min and washed 3 times with 50 ml of the same buffer at 4°.

The nuclei were lysed by slowly pipetting into 100 ml of 0.01 M Tris/HCl, 0.2 mM EDTA, pH 7.8, stirring on ice. After stirring for 15 min the chromatin was collected by centrifugation at 10,000xg for 10 min.

The volume of the chromatin pellet plus 2 ml of lysis buffer was estimated by weighing (8-10 ml) then warmed to room temperature (20-22°). Calcium chloride (0.01 volume of 0.1 M) and micrococcal nuclease (0.01 volume of 12,500 units/ml) were added. Clipping by the nuclease was assisted by gentle pipetting with pasteur pipets broken off to varying tip sizes. After a total digestion time of 30 min, the chromatin was cooled on ice and centrifuged at 16,000xg for 30 min. The somewhat cloudy supernatant was saved (on ice), and the pellet was resuspended by pipetting into 3 ml of 0.01 M Tris/HCl, pH 7.8, 1 mM $CaCl_2$, 125 units/ml micrococcal nuclease and warmed to room temperature for 30 min. After centrifugation as above, the second supernatant was combined with the first. At this stage about 5% of the starting DNA remained with the pellet and could be recovered by a third wash, without further digestion. To avoid dilution of the chromatin this third wash was discarded. The chromatin solution was adjusted to 2 mM EDTA and clarified further by centrifugation at 46,000xg for 30 min.

Removal of histones H1 and H5 was carried out at 0.05 M NaCl with CM-Sephadex C-25-120. For each ml of chromatin, 30 mg of dry CM-Sephadex was added with stirring followed by 0.01 volume of 5 M NaCl added very slowly. Stirring was continued for 60 minutes (at 0°). The resulting suspension was poured into a 0.8 x 12 cm column of additional CM-Sephadex (at room temperature) equilibrated with 0.01 M Tris/HCl, 0.2 mM EDTA, pH 7.8, 0.05 M NaCl. Settling of the ion-exchanger sometimes results in a drop in flow rate which can be corrected by periodic resuspension of the top portion of the column during sample application. The chromatin was washed through with the same buffer, and the A_{260} absorbing samples collected and dialysed overnight against 500 ml of 0.02 M Tris/HCl, 0.2 mM

EDTA, pH 7.8 at 4°. At 0.05 M NaCl, the CM-Sephadex appears to extract micrococcal nuclease as well as H1 and H5 since, after dialysis, no digestion occurs if calcium is added and the chromatin warmed at 37° for one hour. This observation is consistent with the high affinity of micrococcal nuclease for phosphocellulose (13). No H1 or H5 was detected in the chromatin after this step; protein eluted from the CM-Sephadex with 2 M NaCl included primarily H1 and H5 with a very low level of the inner histones, H2a and H2b.

The digestion to core particles was done in siliconized glass tubes to minimize possible binding of micrococcal nuclease to glass (14). A preliminary digestion was used to determine the optimum digestion period; under the conditions described here the optimum time was 25-35 min.

The dialysed chromatin was centrifuged at 16,000xg for 20 min and adjusted to 1 mM $CaCl_2$. After warming to 37° the digestion was started by adding 0.01 volume of 12,500 units/ml micrococcal nuclease. The digestion was stopped by addition of EDTA to 2 mM.

The solution was concentrated about 2-fold by dialysis against 200 ml of 0.01 M Tris/cacodylate, 0.2 mM EDTA, pH 7.2, 0.35 M NaCl, 50% glycerol for 1 hour and then underlayered onto a 1.25 x 120 cm column of Bio-Gel A-1.5m equilibrated with 0.01 M Tris/cacodylate, 0.2 mM EDTA, pH 7.2, 0.2 mM DTT, 0.35 M NaCl. The core particles were eluted at 8 ml/hr while collecting 2 ml samples. These latter steps were carried out at room temperature.

The resulting core particles after dialysis against 0.01 M Tris/cacodylate, 0.2 mM EDTA, pH 7.2, could be stored at 4° for at least 3 months without apparent degradation of the histone or DNA components as judged by electrophoresis.

RESULTS AND DISCUSSION

The preparation of nucleosome cores as described here was designed to give a high yield of DNA in the form of core particles. At each step in the preparation the chromatin concentration is maintained high for ease of handling, speed of processing and efficient use of micrococcal nuclease. We have used chromatin rather than nuclei as a starting material

in order to maximize the yield of soluble chromatin and to avoid the use of detergents (15) or of polyamines such as spermidine (16). Contamination of the chromatin or core particles with the UV-absorbing, strongly fluorescent detergent Nonidet P-40 or Triton X-100 would interfere with fluorescence measurements. Polyamines are known to bind strongly to chromatin and may interfere with nuclease digestion (17); bound to core particles, polyamines can be expected to significantly affect their physical properties. Furthermore, polyamines are known to activate (as well as inhibit) micrococcal nuclease digestion in the absence of calcium ions (18).

Direct digestion of nuclei prepared as described here gave low yields of soluble chromatin while digestion after lysing the nuclei consistently gave about 80% of the chromatin DNA in soluble form. Gentle mechanical shearing by pipetting is used initially to assist diffusion of the nuclease into the chromatin gel. Although damage to chromatin by extensive mechanical shearing has been reported by Noll, et al (19), mild mechanical shearing does not appear to damage chromatin structure (20).

While the use of CM-Sephadex to remove H1 and H5 from soluble chromatin has not, to our knowledge, been reported previously, this ion exchanger has been reported to remove H1 from crude high mobility group proteins at 0.6 M NaCl (21). Figure 1 illustrates the ability of CM-Sephadex to remove the lysine rich histones from soluble chromatin using a batch technique. Without added NaCl, H1 and H5 were not removed (data not shown). At 0.6 M NaCl, H5 was quickly adsorbed while H1 remained even after 1 hour. Bradbury, et al (22) reported a similar observation with the BioRad ion exchange resin AG-50 WX2 at 0.65 M NaCl. At 0.05, 0.1 and 0.35 M NaCl both H1 and H5 were removed. The apparent relative rates of removal at the different salt concentrations cannot be directly compared, since some of the chromatin precipitates at 0.1 and 0.35 M NaCl (presumably with bound H1 and H5), and redissolves as the H1 and H5 are adsorbed to the ion exchanger. The ability of the lysine rich histones to transfer from chromatin to CM-Sephadex at moderate salt

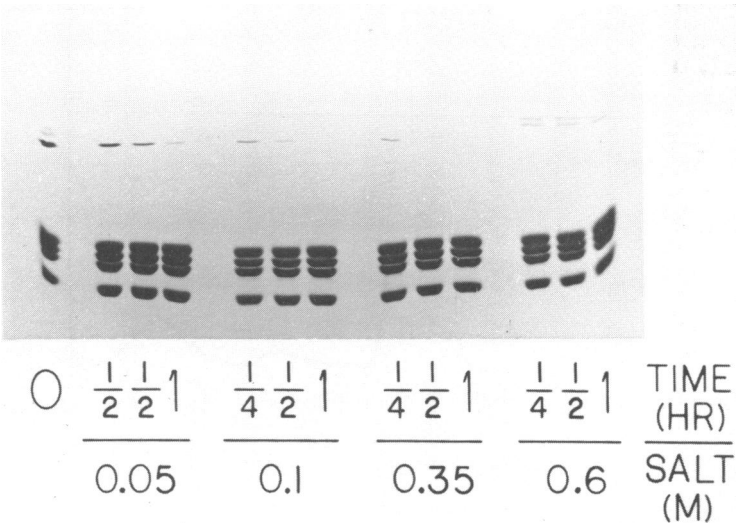

Figure 1. Extraction of histones H1 and H5 from soluble chromatin by CM-Sephadex at various ionic strengths. Chromatin (0.6 ml aliquots, 6 mg/ml DNA) in 0.01 M Tris/HCl, pH 7.8, 1 mM $CaCl_2$, 2 mM Na_2 EDTA was combined with 30 mg CM-Sephadex C-25-120 and allowed to sit on ice for 15 min. Sufficient 5 M NaCl was added with mixing to give the indicated concentrations. The samples were mixed occasionally to resuspend the gel. At the indicated times the samples were centrifuged and a portion of the supernatant removed for SDS disc gel electrophoresis. Electrophoresis was done as described by Laemmli (23) using 3% and 15% stacking and running gels, respectively (both 20:1= acrylamide:bis). The 0.1 x 9 cm x 7 cm long gels were run at 100V for 20 min then at 200V for 40-60 min at 4° and stained with Coomassie blue R.

concentration indicates mobility of the lysine rich histones even at 0.05 M salt. A similar conclusion is suggested by the observation of Ilyin, et al. (24) that H1 rapidly transfers from chromatin to tRNA at low ionic strength.

Generally, chromatography of nucleosome cores has been done on Bio-Gel A-5m or Sepharose 6B (exclusion limit 5 x 10^6) to separate cores from oligomers (15). We have used Bio-Gel A-1.5m (exclusion limit 1.5 x 10^6) in order to take advantage of the somewhat higher resolution and the decreased dilution obtainable with the smaller pore size (Figure 2). The column is run at 0.35 M salt to remove any proteins not bound at this

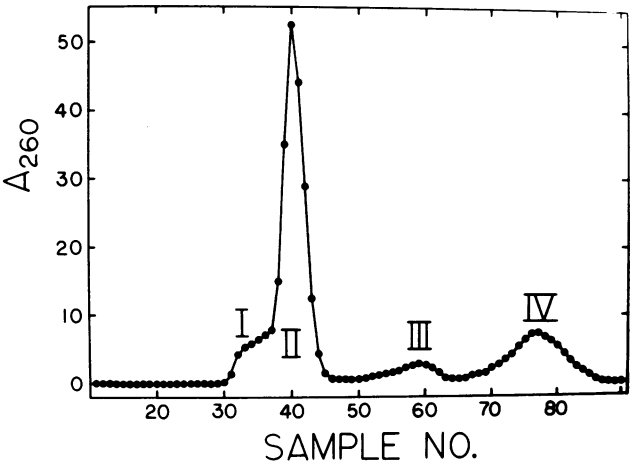

Figure 2. Bio-Gel A-1.5 m chromatographic profile of crude chromatin core particles. See METHODS for details.

concentration. For some purposes, for example preparation of nucleosomes or cores containing HMG proteins (9,25), such a high salt concentration is not desirable.[2] A salt concentration of 0.1 M also gave satisfactory results on Bio-Gel A-1.5m; however, at low salt (0.01 M Tris/HCl, 0.5 mM EDTA, pH 7.5) the monomer core peak moved much closer to the void volume and resolution was lost. For low salt concentration a gel with a higher exclusion limit must be used.[3]

Chromatography on Bio-Gel A-1.5m as described separates the crude nucleosome core preparation into four main peaks of A_{260} as seen in Figure 2. Peak IV elutes at the salt volume, is acid soluble (cold 7% perchloric acid) and shows an 11% hyperchromicity in acid; it is probably mainly mono- and dinucleotides. Electrophoresis of a sample from peak III did not give a DNA band on a 6% gel which should resolve DNA species down to 30 nucleotides in length; also, no protein was detected by electrophoresis or in the UV spectrum. Peak III was acid soluble, but showed 36% hyperchromicity in acid and, considering the discrete peak observed, probably corresponds to short pieces of nucleic acid. Peak II is nucleosome cores and was essentially free of oligomers

beyond sample number 38. Peak I consists mainly of dimer nucleosomes with small amounts of higher oligomers and some core particles with DNA longer than 140 bp.

Figure 3 indicates the purity of the protein and DNA components in the core particle preparation. Much heavier loadings of the gels revealed a very low level of high molecular weight proteins in addition to the histones and of DNA degraded below 140 bp. Comparison of the native DNA mobility with Hae III restriction fragment of PM2 DNA gave an estimate of size for the core DNA of 145 bp. This value is in agreement with recent estimates (5,26).

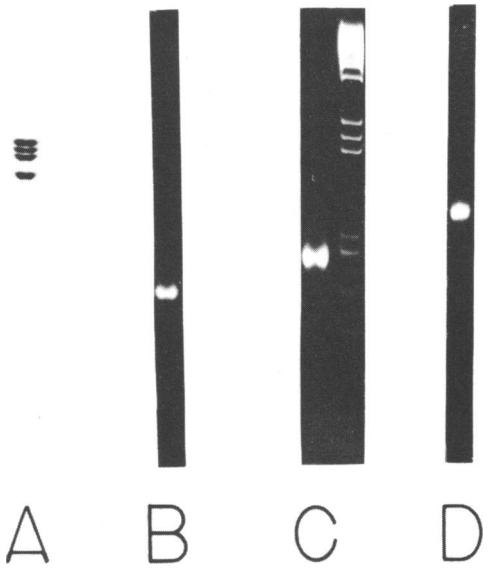

Figure 3. Electrophoresis results for the purified chromatin core particles. All gels were 20:1 acrylamide: bis and 0.1 x 9 cm x 7 cm long. (A) Histones: see Figure 1 for details. (B) Nucleohistone complex was run on 4% gels containing 0.01 M Tris/HCl, 0.5 mM EDTA, pH 8.0 at 70V, 9 mA for 80-90 min. (C) Native DNA was run on 6% gels (27) at 60V, 18 mA for 1.5-2 hours. A standard containing Hae III restriction fragments of PM2 DNA is included. (D) Denatured DNA was run on 6% gels (28) at 100V, 10 mA for 1.5-2 hours. Samples were denatured by dilution with an equal volume of 0.2 N NaOH, 10 M urea, 2 mM EDTA and 20% glycerol (29). After staining for 30 min in 1 µg/ml ethidium bromide, the gels were photographed under UV illumination.

About 80% of the DNA in the nuclei was recovered as soluble chromatin. The losses were primarily related to the sticky nature of the chromatin gel and to efforts to maintain a high chromatin concentration, and should not indicate differential solubilization. The overall yield of DNA applied to the Bio-Gel A-1.5m column was 60-70%. The DNA in the core particle peak represented 35-40% of the starting DNA which corresponds to a 50-60% yield of the possible chromatin core particles (assuming all of the DNA was in the form of 146 bp nucleosomes with 64 bp linkers). This result compares well with the yield of 20% reported by Lutter (2). Sollner-Webb, et al. report a somewhat higher yield (about 60%) of core particles; however, their preparation contained about 20% of 160 bp of DNA (3).

Relative amounts of core and linker DNA can be estimated from the data in Figure 2. The only steps in the preparation likely to result in specific losses of core or linker DNA are the two dialysis steps. When corrected for measured dialysis losses, the A_{260} profile shown in Figure 2 should be representative of the relative amounts of core and linker DNA in the starting chromatin. Table I lists the total A_{260} absorbance units in each of the regions of the chromatogram. Dialysis losses were incorporated into region IV; regions III

Table I. A_{260} units in various fractions obtained from Bio-Gel A-1.5m chromatography of chromatin core particles.[a]

Region	Fraction numbers	Total A_{260} units	% of DNA in core	% of DNA in linker
I	28-37	78	9.9	2.1
II	38-45	387	59.5	-
III	46-65	52[c]	-	8.0
IV	66-85[b]	133[c]	-	20.5
Total		650	69.4	30.6

[a] From Figure 2.

[b] Also includes dialysis losses (23 units).

[c] Corrected for hyperchromicity (see footnote 4).

and IV were corrected for hyperchromicity.[4] The peak I DNA is divided into linker and core DNA assuming that the fraction contains mainly dimer nucleosomes (one 64 bp linker and two 146 bp cores). Using the value of 69.4% for the proportion of DNA in the form of core particles and taking a core DNA length of 146 bp (5,26), the non-core DNA can be estimated to average 64 bp per core particle. The sum (an average of 210 bp of DNA per core particle) is in good agreement with reported estimates for the average nucleosome (core plus linker) DNA repeat length of 207 (30), 212 (31), and 216 (32) bp. This agreement indicates that essentially all of the chicken erythrocyte DNA is folded into nucleosome structures with an average repeat length of 210 bp.

Transitions at very low ionic strength (<10 mM) have previously been reported for core particles from chicken erythrocytes (7,8,10) and from calf thymus (9,11). The erythrocyte core particles were studied by sedimentation (7,8), quasielastic light scattering (7,8) and circular dichroism (8) all of which showed two transitions -- transition 1 at about 1 mM and transition 2 at about 7.5 mM ionic strength. Both transitions could be eliminated by crosslinking with formaldehyde (7) or with dimethylsuberimidate (8). At least one of the transitions was essentially lost on lowering the temperature from 20° to 10° (8). Dieterich, et al (10) studied reconstituted erythrocyte core particles by observing changes in the fluorescence of a covalent probe attached to histone H3. They noted a single transition below 10 mM centered at about 1 mM ionic strength.

Calf thymus cores were studied by transient electric dichroism (9), sedimentation (11), and "contact site" protein crosslinkers (11). The core particles in the latter two reports (11) contained DNA ranging in length up to 180 bp while the former (9) are reported to contain homogeneous 140 bp DNA. Electric dichroism (9) indicates a transition, preventable by dimethylsuberimidate crosslinking, centered at 1.3 mM ionic strength and ranging from 0.3 to 3.0 mM. The transition was characterized by changes in the reduced dichroism, dipole moment and rotational relaxation time.

The reduced dichroism changes were independent of temperature from 0 to 25°. Martinson, et al (11) noted changes in core particle sedimentation coefficient up to about 5 mM. These changes were correlated with crosslinking studies which showed that, at low ionic strength, a contact between H2b and H4 is lost.

Tyrosine fluorescence is highly sensitive to histone renaturation, aggregation and cross-complexing (33-35). We have found that transitions of core particles induced by salt or urea can be readily detected by measurements of fluorescence intensity (F) and anisotropy (R) as illustrated in Table II. Large increases in F are observed above 1 M NaCl, a condition which is known to favor dissociation of histones from DNA (36). Decreases of R are observed at ~1 M NaCl which is known to favor disruption of certain histone-histone interactions (37). Core protein, separated from the DNA, gives R values at 1.2 and 4 M NaCl which are very similar to those for core particles. R also decreases at 5 M urea which causes disruption of histone secondary structure (38). Thus, tyrosine

Table II. Effect of ionic strength and urea concentration on core particle tyrosine fluorescence intensity and anisotropy.

ionic strength (M)	urea (M)	F/F_o[a,b]	R[a]
0.0001	0	1.3	0.149
0.001	0	1.2	0.168
0.01	0	1.15	0.171
0.1	0	1.0	0.173
1.2	0	1.8	0.146
4	0	2.4	0.168
0.015	2.5	1.2	0.165
0.015	5	1.4	0.125
1.0	5	2.2	0.080

[a] Solutions contained NaCl, urea and Tris/HCl, pH 7.5 at various concentrations and core particles at 0.1 mg/ml (0.5 μM) in 3x3 mm cuvettes.

[b] F_o is the fluorescence intensity of an equivalent sample at 0.1 M ionic strength without urea.

fluorescence provides information principally on the core protein and on DNA histone interactions, complementing techniques such as sedimentation and light scattering, which relate to the overall shape of the particle, or reduced dichroism and circular dichroism, which provide information principally on the shape or conformation of the DNA.

The effect of very low ionic strength on R and F of core particles is included in Table II. A relatively small increase in F occurs at 0.1 mM as compared to 0.1 M while the value of R at 0.1 mM is low and similar to that observed at 1.2 M NaCl. These results suggest that very low ionic strength disrupts histone-histone interactions or secondary structure but has little effect on histone-DNA interactions. The transition was found to be reversible on raising the ionic strength from 0.03 to 10 mM.

The effect of low ionic strength on R is illustrated in more detail in Figure 4. A single resolved transition was found to occur below 1 mM ionic strength. The possibility that the fluorescence measurements are insensitive to transition 2 (7,8) was checked by measuring the sedimentation value and anisotropy for the same series of samples. Results are

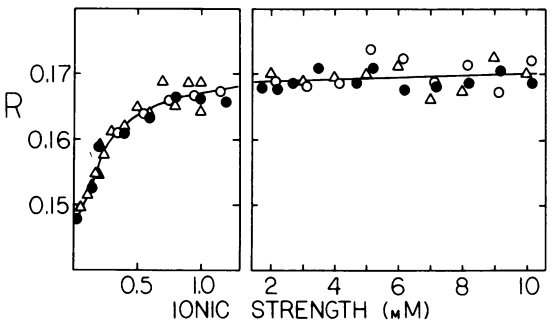

Figure 4. Effect of ionic strengths below 10 mM on the tyrosine fluorescence anisotropy (R) of chicken erythrocyte core particles. Measurements were made on solutions containing 0.1 mg/ml core particles buffered with Tris/HCl (pH 7.5 at 5 mM). Results are given for NaCl (Δ, including 0.03 mM Tris/HCl), KCl (o, including 0.15 mM Tris/HCl) and Tris/HCl only (●) as ionic species. The ionic strengths given should be accurate to within 0.1 mM (see MATERIALS AND METHODS).

given in Fig. 5. Both parameters indicate a single transition occurring below 1 mM. Fluorescence intensity changes also reflect the transition at very low ionic strength; however, they are less useful because of the sensitivity of F to small dilution errors.

Our results differ from those reported by others in two ways. We do not see a transition 2 near 7 mM ionic strength and we observe transition 1 at a lower value of ionic strength.

The absence of transition 2 is probably related to the homogeneous 146 bp DNA in the core particles prepared by the methods described here. Gordon, et al (8) noted that this transition is markedly depressed for core particles of uniform 146 bp DNA. It has been suggested (39,40) that core particles in the absence of H1 or H5 may transiently protect up to 168 bp of DNA from digestion by micrococcal nuclease. Such protection implies at least weak binding of the DNA in excess of 146 bp to the core protein. Thus transition 2 might correspond to breaking of the excess-DNA-histone interactions, perhaps due to increased electrostatic repulsion from the more strongly bound DNA. "Protruding" from the core particle at low ionic strength, this excess DNA should

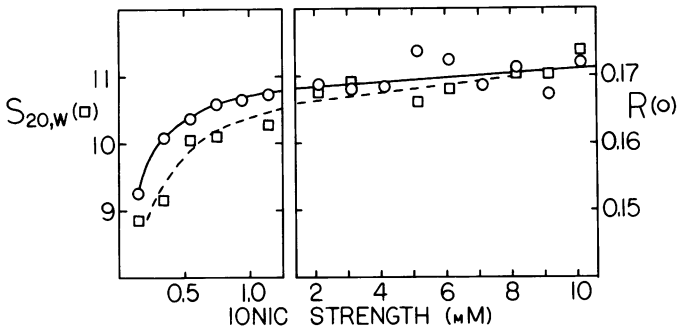

Figure 5. Effect of ionic strength on the sedimentation coefficient of chicken erythrocyte core particles. Samples contained 0.1 mg/ml core particles, 0.15 mM Tris/HCl (pH 7.5 at 5 mM) and KCl to give the indicated ionic strengths. Tyrosine fluorescence anisotropies obtained from aliquots of the same samples used for sedimentation are included for comparison.

decrease both $S_{20,w}$ and the translational diffusion coefficient as is observed (7,8). Also, effects on the circular dichroism spectrum (8) are to be expected.

Transition 1 has been observed to occur near 1 mM ionic strength for both chicken erythrocyte (7,8,10) and calf thymus (9) core particles. In contrast, figures 4 and 5 show a transition centered near 0.2 mM. It is possible that the methods used here to prepare core particles (e.g., by eliminating exposure to high salt to remove H1 and H5) may result in particles of greater stability than those prepared by other methods. However, a number of other possibilities may be suggested. The possibility that the different techniques used may be measuring different aspects of the same transition seems unlikely in view of the comparison of results by sedimentation and tyrosine fluorescence anisotropy shown in Figure 5. We have checked the different salts (Tris-HCl, NaCl, KCl) used by others and have found no effect on the range of ionic strength for the transition (Figure 4). The higher range obtained for erythrocyte core particles may be related to either the presence of fluorescence probes (10) or to the presence of DNA in excess of 146 bp (7,8 and footnote 5) in the particles. We have examined calf thymus core particles prepared essentially as described here; they were found to undergo transition 1 over the same range of ionic strength as reported here for erythrocyte core particles (data not shown). Thus species differences do not account for the higher transition range observed for calf thymus core particles (9).

CONCLUSIONS

Core particles prepared by the methods described here undergo a single defined transition below 10 mM as measured by sedimentation velocity and intrinsic tyrosine fluorescence anisotropy. Tyrosine anisotropy provides a convenient means for studying structural changes in core particles since measurements can be made quickly on small dilute solutions.

ACKNOWLEDGEMENTS

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FOOTNOTES AND REFERENCES

[1]Abbreviations used are: Bis, N,N'-methylene bis(acrylamide); SDS, sodium dodecyl sulfate; bp, base pair; HMG, high mobility group protein; PMSF, phenylmethanesulfonyl fluoride.

[2]The preparation as described in METHODS may result in losses of HMG proteins at two stages before the Bio-Gel A-1.5m column step. Rabbani, et al. (41) have suggested that use of citrate during the isolation of chicken erythrocytes may cause losses of HMG proteins. Sterner, et al. (21) have purified HMG proteins based on their ability to bind to CM-Sephadex.

[3]Ionic strengths below 50 mM can have drastic effects on the elution volume of purified core particles even on Bio-Gel A-5m. The core particles were found to elute at the void volume (V_o) at 1 mM ionic strength. The elution volume increases to about 1.6 V_o at 10 mM then shows a smaller increase to about 1.9 V_o at 50 to 100 mM. While these results may be related to the low ionic strength transitions which have been previously reported (7-9) the possibility also exists that they result from electrostatic repulsion between the negatively charged core particles and the residual carboxylate and sulfate groups which are found in such agarose gels (42).

[4]The correction for hyperchromism was based on the observed total hyperchromism of calf thymus DNA digested to varying extents with micrococcal nuclease and adjusted to 7% perchloric acid. Over the range of partial digestion in which precipitation was not a problem, the total hyperchromicity (digestion plus acid) was observed to vary from 1.51 to 1.57. The values in Table I, regions III and IV were corrected upward for the observed hyperchromism in acid, then divided by 1.55 to correct back to DNA.

[5]We have prepared nucleosomes containing DNA up to 180 bp in length by chromatography at 4°, pH 7.5, 0.35 M NaCl of H1, H5-stripped soluble chromatin (see METHODS) on Bio-Gel A-5m. Preliminary fluorescence measurements show a clear transition, centered at 1 mM ionic strength.

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