Quantitative Analysis of Macromolecular Conformational Changes Using Agarose Gel Electrophoresis: Application to Chromatin Folding†

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ABSTRACT: Quantitative analysis of chromatin electrophoretic mobility (μ) in agarose gels provides a measure of three structural parameters: average surface electrical charge density, which is proportional to the gel-free μ (μ_0), effective radius (R_e), and particle deformability [Fletcher, T. M., Krishnan, U., Serwer, P., & Hansen, J. C. (1994) *Biochemistry 33,* 2226–2233]. To determine whether the intramolecular conformational changes associated with salt-dependent chromatin folding influence these electrophoretic parameters, defined oligonucleosomes were reconstituted from monodisperse tandemly repeated 5S DNA and varying amounts of histone octamers. These oligonucleosomes were subjected to both quantitative agarose gel electrophoresis and analytical velocity ultracentrifugation in buffers containing 0–2 mM $MgCl_2$. Ionic conditions that caused a 40% increase in the oligonucleosome sedimentation coefficient ($s_{20,w}$) also caused both a 30% decrease in R_e and a 60% decrease in the magnitude of the μ_o. Furthermore, the Mg^{2+}-dependent changes in $s_{20,w}$, R_e, and μ_o each exhibited the same nonlinear dependence on the degree of nucleosome saturation of the DNA. These data demonstrate that quantitative agarose gel electrophoresis can be used to detect and characterize the process of chromatin folding. In addition, they suggest that this approach can be used for characterization of the conformational dynamics of many other types of macromolecular assemblies, including those systems that are not yet amenable for study by more traditional quantitative biophysical techniques.

Nucleosomal arrays, which consist of histone octamer–DNA complexes spaced at ~200-bp[1] intervals along the DNA, constitute the initial level of chromatin organization. Nucleosomal arrays are dynamic macromolecules that fold into compact structures in the presence of both monovalent and divalent cations (Thoma et al., 1979; Hansen et al., 1989; Schwarz & Hansen, 1994). Folding of nucleosomal arrays functions to package DNA in the nucleus [see van Holde (1988), Widom (1989), and Wolffe (1992) for reviews]. More recently, in vitro folding of nucleosomal arrays also has been shown to inhibit both transcription initiation and elongation by a eukaryotic RNA polymerase (Hansen & Wolffe, 1992, 1994). Furthermore, both a reduction in the extent of folding and an enhancement in the rate of transcription are observed for nucleosomal arrays that are partially depleted of histone octamers (Hansen & Wolffe, 1992) or arrays that are deficient in H2A/H2B dimers (Hansen & Wolffe, 1994). On the basis of these results, it has been hypothesized that in vivo changes in the core histone content of chromatin influence eukaryotic gene expression in part by modulating the extent of chromatin folding (Hansen & Wolffe, 1994). To test both this and related hypotheses, it has become necessary to develop experimental techniques that can determine the state of folding of functionally interesting regions of native chromatin, e.g., promoters, enhancers, and origins of replication.

Chromatin folding typically has been studied with techniques such as electron microscopy, small-angle X-ray scattering, electric dichroism, and analytical ultracentrifugation (van Holde, 1988; Widom, 1989). However, because these techniques generally require milligram quantities of purified chromatin, they are not useful for analysis of small amounts of specific regions of native chromatin. To provide a technique suitable for this purpose, we have recently been developing agarose gel electrophoresis for use as a quantitative probe of chromatin structure (Fletcher et al., 1994). This approach involves analysis of the electrophoretic mobility (μ) of chromatin using an equation empirically derived by Griess et al. (1989):

$$\mu/\mu'_o = (1 - R_e/P_e)^2 \quad (1)$$

The μ'_o is μ extrapolated to a gel concentration of 0% agarose; after correction for electroosmosis, the μ'_o is converted to the gel-free μ (μ_o). R_e is the effective radius of the chromatin particle, and P_e is the radius of the effective gel pore at a given agarose gel concentration. Several types of information are obtained from this analysis. The μ_o is a measure of average electrical surface charge density (Shaw, 1969). The dependence of R_e on P_e provides a measure of particle deformability. For a comparatively deformable particle such as DNA, as P_e approaches R_e, asymmetrical stretching occurs during gel electrophoresis and the stretched particles migrate end-first (reptate) through the gel. Reptation causes a decrease in R_e with decreasing P_e (Fletcher et al., 1994). In contrast, physiologically spaced nucleosomal arrays in low salt buffer are relatively undeformable and do not reptate as R_e approaches P_e (Fletcher et al., 1994). Finally, when electrophoresis is performed in dilute gels (such that $P_e \gg R_e$), the R_e of linear DNA fragments progressively decreases as

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[1] Abbreviations: μ, electrophoretic mobility; μ_o, gel-free μ; P_e, radius of effective gel pore; R_e, effective radius; r, moles of histone octamer per mole of 208-bp DNA; N, number of nucleosomes bound per 208-12 DNA; bp, base pairs; EDTA, ethylenediaminetetraacetate disodium salt; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; E, 40 mM Tris-HCl (pH 7.8) and 0.25 mM Na_2EDTA; TAE, 40 mM Tris–acetate (pH 7.8) and 0.25 mM Na_2EDTA.

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histone octamers are progressively added onto the DNA (Fletcher et al., 1994). This suggests that, under appropriate conditions, the R_e may provide a measure of macromolecular conformational changes.

Our previous electrophoretic studies were performed exclusively with unfolded nucleosomal arrays in low ionic strength buffer (Fletcher et al., 1994). To determine whether quantitative agarose gel electrophoresis can be used to characterize the intramolecular conformational changes associated with salt-dependent chromatin folding, we determined both the μ and the P_e dependence of the R_e of defined-length nucleosomal arrays as a function of the concentration of $MgCl_2$. In addition, the sedimentation coefficients of the same samples were determined using analytical velocity ultracentrifugation. Results indicate that the salt-dependent changes in electrophoretic parameters closely parallel the salt-dependent changes in sedimentation coefficient that previously have been shown to accompany chromatin folding.

EXPERIMENTAL PROCEDURES

Materials. Whole chicken blood was obtained from Pel Freeze (Rogers, AR) and used as the source of chicken erythrocytes. Histone octamers were isolated from chicken erythrocyte nuclei as described previously (Hansen et al., 1989). The 208-12 DNA template used for the oligonucleosome reconstitutions consists of 12 tandem 208-bp repeats of *Lytechinus* 5S rDNA (Simpson et al., 1985). The 208-12 DNA was purified from the plasmid pPOL208-12 as described previously (Schwarz & Hansen, 1994). The spherical bacteriophage T3 (with a radius of 30.1 nm) was purified by procedures described previously (Serwer et al., 1983). The agarose used was low electroosmosis (LE) agarose, obtained from Research Organics (Cleveland, OH). All chemicals were of reagent grade.

Reconstitution of Saturated and Subsaturated Nucleosomal Arrays. Nucleosomal arrays were reconstituted from purified histone octamers and DNA using the salt dialysis protocol described by Hansen and Lohr (1993). The final dialysis was against 10 mM Tris-HCl and 0.25 mM EDTA, pH 7.8 at 4 °C. The degree of nucleosome loading was controlled by varying r, the moles of histone octamer per mole of 208-bp DNA, from 0.3 to 1.2. After reconstitution, oligonucleosomes were subjected to analytical velocity ultracentrifugation to obtain both the integral distribution of sedimentation coefficients and the average number of nucleosomes bound per DNA molecule (N) as described by Hansen and Lohr (1993). Oligonucleosomes subsequently were dialyzed against E buffer (40 mM Tris-HCl, pH 7.8, 0.25 mM Na_2EDTA), containing 0.1 mM phenylmethanesulfonyl fluoride and varying amounts of $MgCl_2$ for ≥3 h at 4 °C.

Analytical Velocity Ultracentrifugation. Boundary sedimentation velocity studies were performed in a Beckman XL-A analytical ultracentrifuge equipped with scanner optics as described previously (Fletcher et al., 1994). Data were analyzed by the method of van Holde and Weischet (1978). Average sedimentation coefficients were derived from the rate of sedimentation at the boundary midpoint, i.e., the sedimentation coefficient obtained at cumulative fraction = 0.5 of the integral distribution plot.

Quantitative Agarose Gel Electrophoresis. Determination of μ, μ_o, R_e, and P_e was accomplished by use of multigels as described in Fletcher et al. (1994). The μ's were obtained in 0.2–3.0% agarose running gels, each cast within a 1.5% framing gel. Either a 9- or 18-lane template was used to form the multigels. Both the frame and running gels were cast in E buffer containing 0–2.0 mM free $MgCl_2$. Ten-microliter samples containing 0.5–1.0 μg of bacteriophage T3 and 0.6 μg of either DNA or nucleosomal arrays were loaded in each well. Electrophoresis was performed at 1 V/cm for 8–10 h. The temperature of electrophoresis was 24 ± 3 °C, except for the determination of the μ_o of T3, during which it was 24 ± 0.5 °C [see Fletcher et al. (1994)]. Running buffer was circulated throughout the experiment at a rate sufficient to prevent formation of either pH or ion gradients. Multigels were stained first with ethidium bromide and then with Coomassie blue. The stained gels were photographed and digitized. Migration (in centimeters) was measured from the well to the center of a band by use of the NIH IMAGE software (O'Neill et al., 1989). Extrapolation of the linear region of a semilogarithmic plot of μ vs agarose percentage (Ferguson plot) yielded the μ'_o. For DNA and nucleosomal arrays, the μ'_o was converted to μ_o after correction for EEO (μ_E) and normalization by use of T3 as described in Fletcher et al. (1994). The μ_o of T3 in E buffer was -0.789×10^{-4} cm^2/(V·s) and in E buffer + 2 mM $MgCl_2$ was -0.643×10^{-4} cm^2/(V·s). The μ_E of LE agarose in E buffer was 1.07×10^{-5} cm^2/(V·s) and in E buffer + 2 mM $MgCl_2$ was 0.97×10^{-5} cm^2/(V·s). The procedure of normalization of the μ_o of DNA and nucleosomal arrays to the μ_o of T3 is essential for the accuracy of comparisons of μ_o values made here. Values of P_e were calculated from the μ, μ'_o, and the known radius (30.1 nm) of bacteriophage T3 by use of eq 1. Values of R_e were calculated from experimentally determined values of μ, μ'_o, and the P_e, by use of eq 1. The value of P_e had been determined from the μ value of T3 in the same gel used for determining the R_e of either DNA or nucleosomal arrays.

RESULTS

Mg^{2+}-Dependent Changes in the Ferguson Plots of 208-12 DNA and Saturated Nucleosomal Arrays. Saturated 208-12 nucleosomal arrays (12 nucleosomes/DNA) exist in an unfolded beads-on-a-string conformation in low salt buffers such as TAE (Hansen et al., 1989; Fletcher et al., 1994). As demonstrated by both electron microscopy (Thoma et al., 1979) and analytical velocity ultracentrifugation (Schwarz & Hansen, 1994), saturated nucleosomal arrays fold significantly in the presence of $MgCl_2$. Consequently, we initially determined the influence of $MgCl_2$ on the μ of both nucleosome-free 208-12 DNA and saturated 208-12 nucleosomal arrays in 0.2–3.0% agarose. Because the extent of oligonucleosome folding was less in Tris–acetate buffer than it was in Tris-HCl buffer of comparable ionic strength and pH (data not shown), the electrophoresis running buffer used for these experiments was E buffer.

The shapes of the Ferguson plots of 208-12 DNA and nucleosomal arrays in E buffer (Figure 1A) were indistinguishable from those obtained previously in TAE buffer (Fletcher et al., 1994); the plot for saturated 208-12 nucleosomal arrays was convex, while the plot for nucleosome-free 208-12 DNA was linear (Figure 1A). Two points of intersection were observed: ~0.9% and 3.0% agarose. Addition of 2 mM $MgCl_2$ to E buffer had a significant effect on the results. For both the DNA and the nucleosomal arrays, the magnitude of μ in E buffer + 2 mM $MgCl_2$ was lowered at all agarose concentrations (compare panels A and B of Figure 1). In addition, although the shapes of the two Ferguson plots in E buffer + 2 mM $MgCl_2$ were similar to those in E buffer, there was only a single point of intersection in the presence of 2 mM $MgCl_2$–~1.3% agarose.

Correlation between Mg^{2+}-Dependent Changes in the R_e and the $s_{20,w}$ of 208-12 Nucleosomal Arrays. The differences

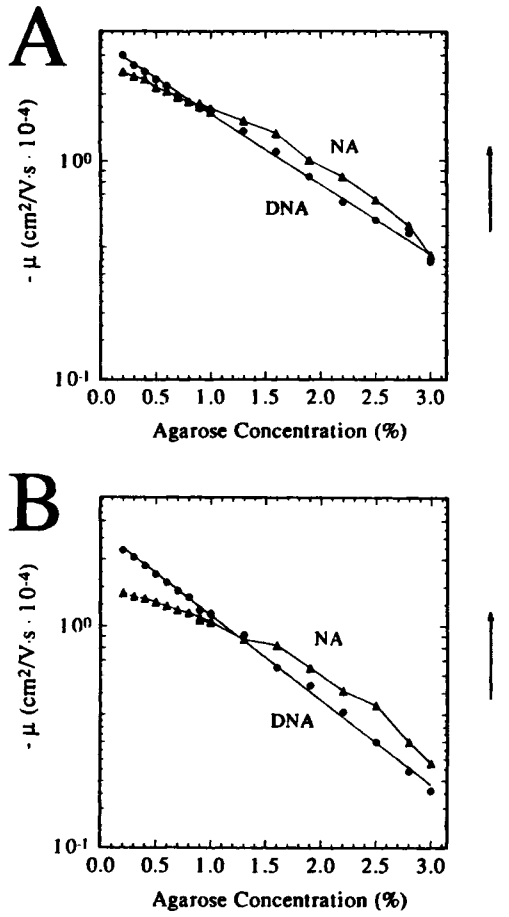

FIGURE 1: Ferguson plots. The μ values of saturated (N = 12) 208-12 nucleosomal arrays (▲) and nucleosome-free 208-12 DNA (●) were determined as described under Experimental Procedures. Each Ferguson plot was generated from two partially overlapping multigels with agarose percentages ranging from 0.2% to 1.0% and 0.9% to 3.0%. DNA refers to nucleosome-free 208-12 DNA, and NA refers to saturated nucleosomal arrays. The running buffer was either E buffer (A) or E buffer + 2 mM $MgCl_2$ (B).

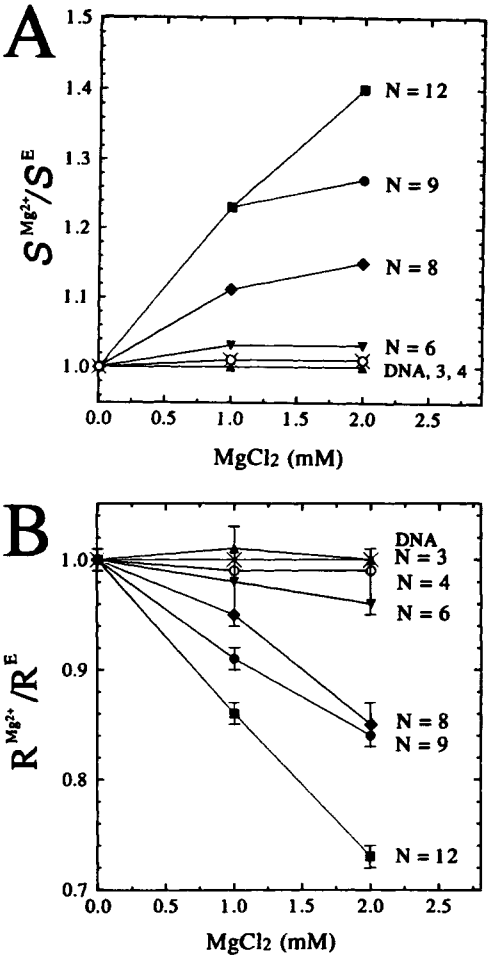

FIGURE 2: Correlation between Mg^{2+}-dependent changes in the $s_{20,w}$ and the R_e of 208-12 nucleosomal arrays. (A) Analytical velocity ultracentrifugation. Nucleosome-free 208-12 DNA (▲) and either N = 3 (×), N = 4 (○), N = 6 (▼), N = 8 (◆), N = 9 (●), or N = 12 (■) 208-12 nucleosomal arrays were sedimented in E buffer ± 2 mM $MgCl_2$ and analyzed as described under Experimental Procedures. Shown for each $MgCl_2$ concentration is the ratio of $s_{20,w}$ in E buffer + $MgCl_2$ divided by the $s_{20,w}$ in E buffer ($s^{Mg^{2+}}/s^E$). The $s_{20,w}$ values were reproducible within 2%. (B) Quantitative agarose gel electrophoresis. The same oligonucleosome samples from panel A, indicated by the same symbols, were electrophoresed in agarose gels having $P_e \geq 200$ nm (≤0.6% agarose) and analyzed as described under Experimental Procedures. Shown for each $MgCl_2$ concentration is the ratio of the R_e in E buffer + $MgCl_2$ divided by the R_e in E buffer ($R^{Mg^{2+}}/R^E$). Each R_e represents the mean ± standard error (SE) of 10 to 15 determinations. The standard error associated with each $R^{Mg^{2+}}/R^E$ ratio was calculated according to the equation % SE of $(R^{Mg^{2+}}/R^E)$ = $[(\%\ SE\ of\ R^{Mg^{2+}})^2 + (\%\ SE\ of\ R^E)^2]^{1/2}$.

among the Ferguson plots shown in Figure 1 are a qualitative indication of Mg^{2+}-dependent changes in the R_e and (or) μ_o of the 208-12 DNA and nucleosomal arrays. To determine whether this altered electrophoretic behavior resulted from chromatin folding, the influence of $MgCl_2$ on the R_e and μ_o of 208-12 nucleosomal arrays was quantified and compared with the influence of $MgCl_2$ on the $s_{20,w}$ measured under identical buffer conditions.

For a saturated (N = 12) nucleosomal array, the ratio of the $s_{20,w}$ in E buffer + 2 mM $MgCl_2$/the $s_{20,w}$ in E buffer progressively increased above 1.0 as the concentration of Mg^{2+} increased to 2 mM (Figure 2A). A ratio greater than 1.0 indicates salt-dependent oligonucleosome folding (Hansen & Lohr, 1993; Schwarz & Hansen, 1994). Saturated nucleosomal arrays in E buffer sedimented as a homogeneous 29S species (data not shown). The 40% increase in $s_{20,w}$ observed in 2 mM $MgCl_2$ for saturated nucleosomal arrays is indicative of formation of an ~40S folded conformation whose extent of compaction is intermediate to that of unfolded 10-nm-diameter and highly folded 30-nm-diameter chromatin (Hansen et al., 1989; Garcia-Ramirez et al., 1992; Schwarz & Hansen, 1994). Subsaturated 208-12 nucleosomal arrays containing 8–9 nucleosomes also experienced significant Mg^{2+}-dependent increases in $s_{20,w}$, although the extent of this increase was reduced compared to that of the saturated arrays (Figure 2A). In contrast, 208-12 nucleosomal arrays containing ≤6 nucleosomes experienced little or no change in $s_{20,w}$ as the $MgCl_2$ concentration was increased from 0 to 2 mM. The Mg^{2+}-dependent folding of saturated and subsaturated 208-12 nucleosomal arrays observed here is comparable to that observed previously in 200 mM NaCl (Hansen & Lohr, 1993).

The influence of $MgCl_2$ on the R_e of the oligonucleosome samples used for Figure 2A was determined in ≤0.6% agarose gels ($P_e \geq 200$ nm). Under these conditions P_e is much larger than R_e, and the R_e of both DNA and nucleosomal arrays is independent of P_e (Fletcher et al., 1994). The R_e of the saturated nucleosomal arrays decreased continuously as the $MgCl_2$ was increased from 0 to 2 mM. In 2.0 mM $MgCl_2$, the R_e decreased by 30%. Consistent with the sedimentation results, subsaturated arrays containing 8–9 nucleosomes also showed a progressive decrease in R_e, although the extent of the decrease was less than that of saturated arrays. Finally, subsaturated arrays containing ≤6 nucleosomes exhibited little, if any, Mg^{2+}-dependent decrease in R_e. Taken together, the data in panels A and B of Figure 2 indicate that the Mg^{2+}-induced decrease in R_e is caused by the Mg^{2+}-induced folding

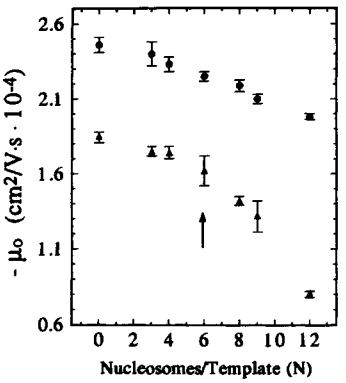

FIGURE 3: Mg^{2+}-dependent changes in the μ_o of 208-12 DNA and nucleosomal arrays. The μ_o values were determined in either E buffer (●) or E buffer + 2 mM $MgCl_2$ (▲). Data were acquired from multigels whose agarose percentage ranged from 0.2% to 1.0% as described under Experimental Procedures. Each point represents the mean ± 1 standard deviation of three to four determinations.

that has previously been shown to also cause an increase in $s_{20,w}$.

The measurement of the oligonucleosome R_e in $MgCl_2$ depends on the assumption that both the radius of bacteriophage T3 and the P_e of the agarose gels are not changed by $MgCl_2$. To determine whether the P_e was altered by the $MgCl_2$ present in the running buffers, the gels were initially cast in E buffer + 2 mM $MgCl_2$ and then soaked for ≥12 h in E buffer (with several buffer changes) to remove the $MgCl_2$. Electrophoresis of bacteriophage T3 subsequently was performed in E buffer. Conversely, in a separate experiment, gels were first cast in E buffer and subsequently soaked and subjected to electrophoresis in E buffer + 2 mM $MgCl_2$. In both cases, the calculated P_e's were the same, within experimental error, as those measured from gels in which both the casting buffer and running buffer was E buffer (data not shown). These controls indicate that the data in Figure 2B were not significantly influenced by Mg^{2+}-dependent changes in either the radius of bacteriophage T3 or the P_e of the agarose gels.

Effect of $MgCl_2$ on the μ_o of 208-12 DNA and Nucleosomal Arrays. In comparison to the μ_o values obtained in E buffer (top plot in Figure 3), the magnitude of the μ_o values obtained in E buffer + 2 mM $MgCl_2$ (bottom plot in Figure 3) was lower for both histone-free DNA and for nucleosomal arrays. The Mg^{2+}-dependent decrease in the magnitude of the μ_o of the histone-free DNA was 25%, compared to 60% for the saturated nucleosomal arrays. As shown previously in TAE buffer (Fletcher et al., 1994), the dependence of μ_o on N in E buffer was linear. However, it was nonlinear in E buffer + 2 mM $MgCl_2$ (arrow, Figure 3); subsaturated arrays containing ≤6 nucleosomes exhibited the same 25% decrease in the magnitude of μ_o as the DNA alone, whereas arrays containing 9 and 12 nucleosomes exhibited 40% and 60% decreases, respectively.

Effect of $MgCl_2$ on the Reptation of 208-12 DNA and Nucleosomal Arrays. In both E buffer (Figure 4A) and the TAE buffer used in a previous study (Fletcher et al., 1994), the R_e for 208-12 DNA fragments containing ≤9 (but not 12) nucleosomes decreased as the P_e decreased from 150 to 40 nm. This behavior is thought to result from particle deformation and reptation. Qualitatively, this behavior was also observed in E buffer + 2 mM $MgCl_2$ for 208-12 DNA fragments containing ≤6 nucleosomes but not for fragments that contained 9 nucleosomes (Figure 4B); only N = 9 nucleosomal arrays were converted from a reptating to a nonreptating particle in 2 mM $MgCl_2$. The R_e of an N = 12 nucleosomal array is independent of P_e in both E buffer and E buffer + 2 mM $MgCl_2$ (Figure 4). Consequently, the Mg^{2+}-dependent decrease in R_e of saturated 208-12 nucleosomal arrays observed in high P_e (Figure 2B) was quantifiable even in very low P_e.

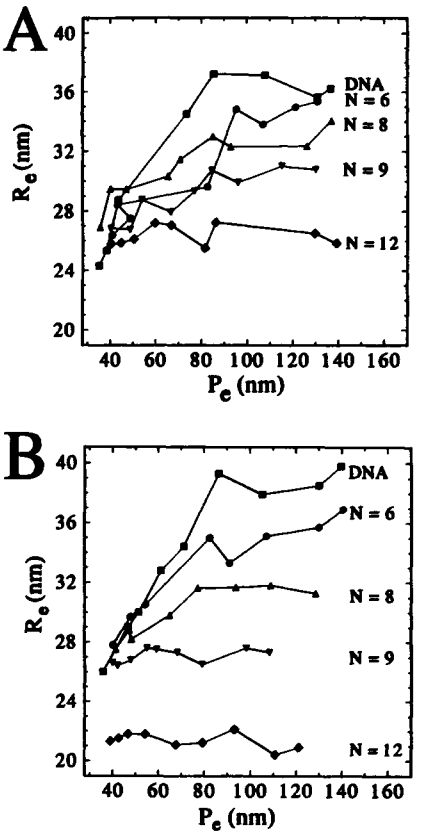

FIGURE 4: P_e dependence of the R_e of 208-12 DNA and nucleosomal arrays. The R_e values of nucleosome-free 208-12 DNA (■) and either N = 6 (●), N = 8 (▲), N = 9 (▼), or N = 12 (◆) 208-12 nucleosomal arrays were determined in multigels whose agarose percentage ranged from 0.9% to 3.0% as described under Experimental Procedures. Values of N are also indicated in the figures. DNA refers to nucleosome-free DNA. The running buffer was either E buffer (A) or E buffer + 2 mM $MgCl_2$ (B). The data in panels A and B are representative of the results obtained in two to six separate experiments for each value of N.

DISCUSSION

Saturated 208-12 nucleosomal arrays sediment at 29 S in very low salt buffers (Hansen et al., 1989; Garcia-Ramirez et al., 1992). Under these conditions the nucleosomal arrays adopt an unfolded beads-on-a-string conformation. Using analytical ultracentrifugation analysis, it has been demonstrated that the increased sedimentation coefficients present in both 100 mM NaCl (Hansen et al., 1989; Garcia-Ramirez et al., 1992) and 1–2 mM $MgCl_2$ (Schwarz & Hansen, 1994) result from folding of the nucleosomal array. Moreover, in both NaCl (Hansen & Lohr, 1993) and $MgCl_2$ (Figure 2A) there is a characteristic relationship between the degree of nucleosome saturation and salt-dependent folding; the 208-12 DNA must contain ≥8–9 nucleosomes before the salt-dependent change in sedimentation coefficient is significant. In the present study, we have shown that there are large Mg^{2+}-dependent changes in both the R_e and μ_o of saturated 208-12 nucleosomal arrays under conditions where there is also a large Mg^{2+}-dependent change in $s_{20,w}$. Furthermore, the Mg^{2+}-dependent changes in the oligonucleosome R_e and μ_o had the same dependence on nucleosome saturation as did the Mg^{2+}-dependent changes in $s_{20,w}$ (Figures 2–4). We therefore

conclude that Mg^{2+}-dependent changes in the electrophoretic parameters are caused by folding of the nucleosomal array.

The nucleosomes of a subsaturated 208-12 array are distributed mostly randomly among the 12 5S DNA repeats (Hansen & Lohr, 1993). Thus, only 5–10% of adjacent 5S DNA repeats will be occupied by nucleosomes in a $N = 6$ nucleosomal array, whereas about 60% of the adjacent repeats will be occupied by nucleosomes in an $N = 9$ array. The observation that 8–9 nucleosomes must be bound to have significant Mg^{2+}-induced changes in $s_{20,w}$, R_e, and μ_o demonstrates both that nucleosome occupancy of adjacent 5S DNA repeats is essential for Mg^{2+}-dependent oligonucleosome folding and that folding is locally inhibited if the nucleosomes are separated by ≥270 bp of DNA. These data are consistent with the previous conclusion that a folded 40S 208-12 nucleosomal array most closely resembles a 90° contacting zigzag, in which nucleosome "n" interacts with both nucleosome "$n + 1$" and "$n - 1$" (Garcia-Ramirez et al., 1992). Furthermore, these data help explain why the presence of even a single nucleosome-free region inhibits Mg^{2+}-dependent formation of the 55S solenoidal conformation of a 208-12 nucleosomal array (Schwarz & Hansen, 1994).

For rod-shaped macromolecules, the surface area of the molecule is closely correlated with surface area of the sphere that has the experimentally determined R_e (Griess et al., 1990). This relationship also has been shown for the unfolded 32S (beads-on-a-string) conformation of a 208-12 nucleosomal array (Fletcher et al., 1994). The folded 40S conformation of a 208-12 nucleosomal array that is formed in 2 mM $MgCl_2$ also can be modeled as a rod; for a 12-mer nucleosomal array, a 90° contacting zigzag structure would be best represented by a rod of 22 × 144 nm. The surface area of this rod is 5.7×10^3 nm^2. The surface area of a sphere calculated from the oligonucleosome R_e measured in 2 mM $MgCl_2$ (21 nm) is 5.6×10^3 nm^2. In contrast, folding of a 208-12 $MgCl_2$ nucleosomal array to form two turns of a contacting helix (i.e., a 30-nm-diameter solenoidal particle) would produce a roughly spherical structure that is predicted to both sediment at 52 S [Hansen et al., 1989; see Schwarz and Hansen (1994)] and have an R_e of ~15 nm. Thus, these data support the conclusion that both the electrophoretic analysis and the solution-state sedimentation analysis are characterizing the same zigzag-like folded particle.

The analysis of the μ_o of both nucleosome-free 208-12 DNA and nucleosomal arrays in E buffer ± 2.0 mM $MgCl_2$ (Figure 3) has yielded the following conclusions. (1) The μ_o of the 208-12 DNA molecule in 2.0 mM $MgCl_2$ is 25% lower in magnitude than that in E buffer. Because no decrease in the magnitude of μ_o is observed in 50 mM NaCl (data not shown), elevated ionic strength is not the cause. Rather, this observation is explained by the assumption that the reduction in the magnitude of μ_o in $MgCl_2$ results from binding of the Mg^{2+} to DNA. (2) Independent of N, the μ_o of nucleosomal arrays containing ≤6 nucleosomes also is 25% lower in magnitude than the corresponding values in E buffer. In contrast, there is a 60% decrease in the magnitude of μ_o of saturated nucleosomal arrays in 2.0 mM $MgCl_2$. These observations indicate that, in addition to the primary reduction caused by binding of Mg^{2+} to the DNA, formation of the folded 40S conformation of a saturated 208-12 nucleosomal array causes a large secondary reduction in the average negative electrical surface charge density. Salt-dependent folding of native H1-depleted dinucleosomes is associated with linker DNA bending (Yao et al., 1991). One explanation for the folding-dependent decrease in the magnitude of the μ_o is that the linker DNA is sequestered between the nucleosomes in a folded 40S nucleosomal array and hence effectively removed from the particle surface. Such a location for the linker DNA within H1-stabilized 30-nm chromatin has been proposed by other investigators (McGhee et al., 1983; Bavykin et al., 1990; Zlatanova et al., 1994). Alternatively, folding of the nucleosomal array may alter the structure of the linker DNA such that it binds more Mg^{2+} in the folded state. Regardless of the molecular mechanism(s) involved, these data indicate that one of the most profound changes associated with Mg^{2+}-dependent folding of H1-depleted nucleosomal arrays is a drastic reduction in the negative surface electrical charge density.

In conclusion, the strong correlation between Mg^{2+}-dependent changes in oligonucleosome $s_{20,w}$ and the concomitant changes in R_e and μ_o indicates that quantitative agarose gel electrophoresis can be used as an analytical method for studying chromatin folding. In addition, these studies, together with our previous work performed at low ionic strength (Fletcher et al., 1994), lay the foundation for structural analysis of specific chromatin fragments isolated directly from cell nuclei. Although we have focused exclusively on chromatin, the quantitative electrophoretic approach described in this paper should prove useful for characterization of the structural features and conformational dynamics of many other types of functionally interesting macromolecular assemblies. This is particularly the case for those systems that are not yet amenable for study by more traditional quantitative biophysical techniques.

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