Salt-Induced Release of DNA from Nucleosome Core Particles†

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ABSTRACT: At elevated salt concentrations, the structure of chromatin is destabilized. This paper is concerned with the processes by which DNA is released from nucleosome core particles in free, uncomplexed form. Our experiments indicate that the DNA release reaction has distinctly different characteristics below and above ~0.75 M NaCl. Below this concentration of salt, release of the histones from the DNA is highly cooperative, so that no dissociation intermediates are even seen. Above this salt concentration, histone release is not so cooperative; H2A and H2B are released from the DNA more readily than are H3 and H4. This results in an apparently heterogeneous population of (H2A, H2B)-depleted intermediate species sedimenting at rates between that of free DNA and that of intact core particles. Dissociation of core particles at NaCl concentrations below 0.75 M is readily reversible. Reassociation of DNA and histones from higher salt concentrations is nearly quantitative if carried out by gradual decrease of salt concentration, but rapid dilution to low salt results in the formation of a fraction of metastable nucleosome multimers. To help organize our description of the DNA release process, we introduce a stability diagram for the core particle, defined with respect to the independent variables of salt concentration and particle concentration. We draw upon our own experimental work and also upon the work of several other laboratories. We distinguish five major regions in this diagram.

It is unlikely that the processes of DNA replication, transcription, genetic recombination, and DNA repair can occur in eukaryotes without, at least, a transient breakdown of chromatin structure. To provide a conceptual foundation for understanding these molecular biological processes, the stability of the fundamental subunit of chromatin—the nucleosome—must be investigated. Although a vast amount of research has been done on nucleosomes since their discovery ca. 1973, there still is no *comprehensive* theory of how these particles respond to perturbations in local environment. Such perturbations would include changes in salt concentration, temperature, pH, the addition of small physiological molecules like polyamines or drugs, etc.

A fundamental principle of nucleosome stability is that the DNA is held to the histone core by bonds having significant electrostatic character. Since electrostatic effects can be modulated by salt, the systematic variation of the type and concentration of the salt in the milieu provides a way to examine the bonding which stabilizes the nucleosome. Over the years, there have been claims of as many as nine different types of nucleosome instabilities induced by increases in salt concentration: (1) particle unfolding without histone loss (Dieterich et al., 1977, 1979; Wilhelm & Wilhelm, 1980); (2) the unbinding of the basic "tail regions" of the histones from contacts with the DNA (Cary et al., 1978; Walker, 1984); (3) a rotation of one section of the histone core relative to another section of the histone core (Chung & Lewis, 1986); (4) the release and straightening of the termini of the DNA (Russev et al., 1980; Harrington, 1982); (5) the loss of one or both H2A·H2B dimers (LaRue & Pallotta, 1976; Wilhelm et al., 1978; Jorcano & Ruiz-Carrillo, 1979; Burton et al., 1978, 1979; Oohara & Wada, 1987); (6) the exchange of H2A·H2B dimers between nucleosomes and an exogenous pool (Louters & Chalkley, 1984); (7) the total release of the DNA from a fraction of the particles (Stacks & Schumaker, 1979; Russev et al., 1980; Vassilev et al., 1981; Cotton & Hamkalo, 1981; Eisenberg & Felsenfeld, 1981; Ausio et al., 1984; Yager & van Holde, 1984); (8) the binding of a histone core octamer to the exterior of an intact nucleosome, possibly in a disproportionation reaction (Voordouw & Eisenberg, 1978; Stein, 1979); (9) the sliding of the histone core along the DNA, in particles containing DNA >146 base pairs (bp)[1] in length [Beard, 1978; see also van Holde and Yager (1985)]. In some cases, the multiplicity of results may arise from inconsistencies in nucleosome preparation or differences in solution conditions (temperature, nucleosome concentration) used in the various studies. In other cases, the observed property is not uniquely related to molecular structure, and two or more structural changes can give rise to the same change in the property. In order to unify the disparate results of many studies, it is essential to define a set of underlying principles which govern nucleosome stability as a function of several variables.

In this paper, we have attempted to critically examine *one* of the ways in which nucleosomes respond to elevated salt concentration, e.g., dissociation. We have utilized homogeneous and well-characterized preparations of chicken eryth-

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[1] Abbreviations: bp, base pair(s) (of DNA); EDTA, (ethylenedinitrilo)tetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; PMSF, phenylmethanesulfonyl fluoride; DMSO, dimethyl sulfoxide; CD, circular dichroism; SDS, sodium dodecyl sulfate; BSP1286, restriction endonuclease (type II) BSP 1286; *Cfo*I, restriction endonuclease (type II) *Cfo*I; pBR322, plasmid pBR322; NaOAc, sodium acetate; S, svedberg unit (10^{-13} s); nt, nucleotide(s) (of DNA); UV, ultraviolet.

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rocyte *core particles* containing 144 ± 2 base pairs of DNA and no proteins other than the core histones. Additionally, we have employed a number of investigative techniques, including velocity sedimentation, gel exclusion chromatography, and electrophoresis on polyacrylamide "native" gels. Core particles are examined under a variety of particle concentrations and salt concentrations and are monitored at both short and long times after jumping to elevated salt. Finally, we have used the data to establish a "stability diagram" that describes the state of nucleosome core particles as a function of particle concentration and salt concentration. We examine the reversibility of transitions between various regions in the stability diagram and test for the presence of intermediate states in these transitions.

Materials and Methods

Preparation of Core Particles. Chicken erythrocyte core particles were prepared by two different methods. The two methods give core particles of almost identicial quality.

Method I, basically a modification of that of Lutter (1978), has been described by Paton et al. (1983). This method involves (1) a light nuclease digestion of chromatin in nuclei, (2) pelleting of the nuclei by centrifugation, (3) resuspension and lysis of the nuclei in EDTA, and (4) removal of debris by centrifugation. The yield of core particles from the 50 mL of blood of one chicken is 1–1.5 mL × 100 A_{260} units (50–75 nmol). Core particles prepared by this method were stored at 10 mg/mL and T = 6 °C until use. No preparations over 6 weeks old were used.

Method II is a more radical modification of Lutter's method, which gives an increased yield of very homogeneous core particles. Since we currently find this the most satisfactory method, we describe it in some detail.

(*A*) *Erythrocyte Lysis.* Fresh chicken blood was mixed with heparin to 13 units/mL, filtered through cheesecloth, and centrifuged 4 min at 1000*g*. All subsequent operations (except nuclease digestions) were done at T = 0–4 °C. The erythrocyte pellet was washed 3 times by centrifuging 4 min at 1000*g* through 25 volumes of buffer A (0.34 M sucrose, 15 mM Tris, 60 mM KCl, 15 mM NaCl, 0.5 mM spermidine, 0.15 mM spermine, 2 mM EDTA, 2 mM EGTA, and 15 mM β-mercaptoethanol, pH 7.5) + 0.1 mM PMSF. The pelleted erythrocytes were lysed with a tissue homogenizer in 25 volumes of buffer A + 0.1 mM PMSF + 0.5% Nonident P-40. The nuclei were pelleted with a 5 min, 1000*g* centrifugation, and were then washed twice by centrifugation through 50 volumes of buffer A + 0.1 mM PMSF + 0.5% Nonidet P-40.

(*B*) *First Digestion.* To prepare for the first nuclease digestion, all EDTA and EGTA were removed by washing the nuclei 3 times with a 4-min, 1000*g* centrifugation in 50 volumes of buffer B (0.34 M sucrose, 15 mM Tris, 60 mM KCl, 15 mM NaCl, 0.5 mM spermidine, 0.15 mM spermine, and 15 mM β-mercaptoethanol, pH 7.5) + 0.1 mM PMSF. The nuclei were resuspended in buffer B at a concentration giving A_{260} = 65. The suspension was warmed to T = 37 °C, and $CaCl_2$ was added to 1 mM. *Staphylococcus aureus* (Foggi) nuclease (Sigma) was added to 45 units/mL. After 5 min, the digestion was stopped by adding EDTA to a free concentration of 1.5 mM and lowering T to 0 °C. The nuclei were pelleted with a 5-min, 8000*g* centrifugation. This first digestion generated very long chromatin fragments, which remained in the nuclei during centrifugation.

(*C*) *Lysis of Nuclei.* The nuclei were lysed by resuspension in 5–10 volumes of 0.25 mM EDTA, pH 7.5, followed by gentle stirring for 1 h. Debris was eliminated by centrifuging for 20 min at 8000*g* and discarding the pellet. At this point, approximately 50–75% of the chromatin remained in the supernatant, and the other 25–50% was discarded. The soluble chromatin was diluted to A_{260} = 50 with 0.25 mM EDTA, pH 7.5; 0.01 volume of 1 M Tris, pH 8.0, was then added. The final solution was ~0.25 mM EDTA/10 mM Tris, pH 8.0.

(*D*) *Stripping.* H1, H5, and non-histone proteins were removed according to Libertini and Small (1980). NaCl was added to 0.35 M, and carboxymethyl-Sephadex (Pharmacia) was added to 30 mg/mL. The suspension was stirred gently for 3 h. The cation-exchange resin was then removed by centrifugation for 25 min at 8000*g*.

(*E*) *Second Digestion.* The chromatin was dialyzed into buffer B and then diluted to a concentration of A_{260} = 25. The temperature was raised to 37 °C, $CaCl_2$ was added to 1 mM, and staphylococcal nuclease was added to 50 units/mL. After 10 min, the digestion was stopped by cooling on ice and adding 0.25 M EDTA (pH 8.0) to a free concentration of 1.5 mM. This second nuclease digestion generates about 50% mononucleosomes and about 50% higher nucleosome oligomers; a large fraction of the mononucleosomes contain DNA considerably longer than 146 bp.

To isolate mononucleosomes, the redigested chromatin was concentrated to A_{260} = 100 by ultrafiltration (Amicon PM 30) and then loaded in ≤25-mL batches onto a 175–200 cm × 20 cm^2 Sephacryl S-300 or S-400 gel filtration column (Pharmacia). The column buffer was 20 mM NaCl, 5 mM Tris, 0.2 mM EDTA, and 2 mM β-mercaptoethanol, pH 7.5 (buffer "C"). At a flow rate of 1–2 mL/min, the mononucleosome peak eluted at ~1500 mL. Mononucleosome fractions were examined by gel electrophoresis (see below); those showing DNA lengths less than about 160 bp were discarded, while those showing DNA lengths greater than this limit were all pooled.

(*F*) *Third Digestion.* $CaCl_2$ was added to the mononucleosome pool to a free concentration of 0.8 mM. A test digestion was done to determine conditions which maximized the yield of *core particles* (containing ~146 bp of DNA) while holding the production of subnucleosomal particles (containing <146 bp of DNA) to an acceptable minimum. For an A_{260} ≃ 10 solution of mononucleosomes, we found a 2–15-min digestion by 10 units/mL nuclease, at T = 37 °C, to be acceptable. We digested the mononucleosome pool under these conditions. The digestion was then stopped by adding EDTA to a free concentration of 1.7 mM and cooling to 0 °C.

This third digestion produced "trimmed" mononucleosomes, which were concentrated to A_{260} ≃ 100 by ultrafiltration (Amicon PM 50) and loaded on the Sephacryl column. Fractions were examined by gel electrophoresis, and those containing homogeneous *core particles* were pooled. The pooled core particles were concentrated by ultrafiltration to A_{260} ≃ 10 (Amicon PM 50), dialyzed into 10 mM Tris/0.25 mM EDTA, pH 7.5, and stored on ice. The typical yield from 50 mL of blood was ~100 mL of solution with an absorbance of 10 at λ = 260 nm (~500 nmol of core particles). This corresponds to about 20% of the starting material (core particles *in chromatin*) and is about 7–10 times the yield of method I. No preparations were used that were over 6 weeks old.

Possibility of Fractionation. Each of the above methods for preparing nucleosomes requires a number of centrifugations, chromatography steps, etc. in a variety of different buffers. It is possible that, in one or more of these steps, a fractionation into nucleosome subpopulations may occur on the basis of some physical or compositional difference. Such fractionations have been reported by a number of researchers

(Sanders, 1978; Fulmer & Bloomfield, 1982; Rocha et al., 1984; Ausio et al., 1986). We have not tested for this possibility.

Electrophoresis as a Check on Particle Integrity. Histones were prepared and run on 0.5-mM-thick discontinuous SDS gels (Laemmli, 1971). The stacking and running portions of the gels were 6% and 15% polyacrylamide, respectively [each with a bis(acrylamide):acrylamide ratio = 1:37.5]. Electrophoresis (at $T = 4$ °C) was done at ~25 V/cm for ~90 min. Staining was with Coomassie blue and then with silver (Wray et al., 1981).

Polyacrylamide gel electrophoresis was used to examine core particle samples for the presence of contaminant nucleoprotein species and for free DNA. These gels were designed to allow nucleoprotein particles to remain intact during the electrophoresis; hence, we refer to them as "native" gels. The gels were 0.8 mm thick, 3.5% polyacrylamide [bis(acrylamide): acrylamide ratio = 1:20], and contained a 40 mM Tris, 20 mM NaOAc, and 1 mM EDTA (pH 7.2) buffer (Kovacic, 1976; Kovacic & van Holde, 1977). Core particle samples, made up without any dye present, were electrophoresed into the gels at 3 V/cm and were then run at 15 V/cm. The gels were stained with 0.5 μg/mL ethidium bromide and photographed on a long-wavelength UV transilluminator with Polaroid 667 or 515 film (red filter).

Core particle multimers were examined on another type of native gel, made of 4% acrylamide [1:38 bis(acrylamide): acrylamide ratio] in 90 mM Tris–borate/1 mM EDTA, pH 8. The gels were run at ~20 V/cm and then were stained in 0.5 μg/mL ethidium bromide and photographed.

DNA was purified from core particles and examined both in double- and in single-stranded form. For double-stranded DNA, native gels were prepared and run as above, stained with 0.5 μg/mL ethidium bromide, and photographed. "Single-stranded DNA" gels were prepared by the method of Maniatis et al. (1975), except that no SDS was used, either in the gels or in the running buffer. Purified core particle DNA in 10 mm Tris/0.25 mM EDTA, pH 7.5, was either mixed with DNA markers in this buffer or with the buffer alone and then was made 30% in analytical-grade DMSO, 5% in glycerol, and 0.025% in each of bromophenol blue and xylene cyanol. The samples were heated to 100 °C for 5 min, cooled on ice, loaded onto gels, and run immediately. The voltage gradient was ~4 V/cm initially and was increased to ~25 V/cm as soon as the samples entered the gel. Running temperature was 4 °C. Gels were simultaneously washed free of urea and stained in 0.5 μg/mL ethidium bromide by gentle agitation at 4 °C; they were then photographed.

Sedimentation Coefficient Measurements. A Beckman Model E ultracentrifuge with photoelectric scanner was used to measure sedimentation coefficients. Experiments utilized both 12-mm and 30-mm cells. Most experiments were done at nucleosome concentrations giving $A_{265} \simeq 0.9$ in a 30-mm ultracentrifuge cell. Some experiments, however, were done on samples that were up to 3× more dilute than this or up to 30× more concentrated than this. For the concentrated samples, an appropriately higher wavelength was chosen for the scanner, to give a total absorbance of ~0.9 across the particular ultracentrifuge cell that was used. All experiments were performed at 20.5 ± 1.5 °C. The temperature within a run was controlled to within 0.1 °C. All studies utilized a 10 mM Tris/0.25 mM EDTA buffer to which varying concentrations of NaCl were added (final pH 7.50 ± 0.05). For very dilute solutions, in the absence of added salts, 0.5% sucrose was added to stabilize the boundary.

All core particle sedimentation coefficients were corrected to $s_{20,w}$ using standard corrections for buffer density and viscosity (Weast, 1978). From the data of Olins et al. (1976), Eisenberg and Felsenfeld (1981), and Ausio et al. (1984), we have assumed for core particles an apparent partial specific volume $\phi' = 0.66$ cm^3/g independent of NaCl concentration. For DNA, we have used $\phi' = 0.55$.

To analyze a sedimentation velocity run for the fraction of dissociated material, either of two methods was used. (1) Several scans from the middle portion of the run were measured and were corrected for radial dilution. The percentage of dissociated material in each was calculated. The results were averaged. (2) Some runs were analyzed by the method of van Holde and Weischet (1978) to determine the integral distribution of the sedimentation coefficient. Between five and eight scans were measured in such cases.

In sedimentation velocity runs performed on core particle samples, it often was hard to estimate precisely the percentage of material (free DNA) sedimenting with $s_{20,w} \simeq 5$–6 S. There were three reasons for this difficulty. (1) The inevitable noise in a scanner trace tended to obscure any signal from free DNA that was just slightly above base line. (2) The high pressure in the ultracentrifuge cell appears to induce a small amount of core particle dissociation. The effect is most noticeable during the late part of a run. The effect indicates that the specific volume of the core particle decreases upon dissociation. (3) We have noticed, for core particles at a concentration $A_{260} \simeq 0.2$–0.3 in 10 mM Tris/0.25 mM EDTA (pH 7.5), that if the concentration of sucrose is increased from 0.5% to 1% and then to 2%, there is a progressive decrease in the apparent percentage of slow boundary. This suggests that, at least in salt-free, highly dilute solutions, a component of the slow boundary may be an artifact due to turbulence. This turbulence effect should not complicate the experiments in which NaCl has been added, for the salt will provide a stabilizing density gradient.

Gel Filtration Tests for Partial Dissociation of Core Particles. To test for partial dissociation, a solution of core particles was jumped to elevated salt concentration and loaded onto a Bio-Gel P-100 gel filtration column (nominal exclusion limit $M_r \approx 100\,000$; column dimensions 6.8 × 0.5 cm; loaded volume 100 μL). Intact core particles, histone-depleted particles, free DNA, and nucleosome multimers (if such species were generated) were expected to elute at the void volume; histone tetramers and dimers were expected to elute later. These expectations were based on the nominal exclusion limit of the column material and also on our calibration of the column with native core particles (M_r 204 000), BSA (M_r 64 000), and lysozyme (M_r 14 600; monomer–dimer equilibrium anticipated; Sophianopoulos & van Holde, 1964).

Column fractions were assayed for the A_{220}/A_{260} ratio on a Hewlett-Packard 8450A spectrophotometer. They were also examined on Laemmli-type gels for histone stoichiometry and on native gels for free DNA, an authentic core particle band, and the presence of other nucleoprotein bands.

Method for Generating and Analyzing Nucleosome "Multimers" on Polyacrylamide Gels. Core particles were diluted from a low-salt stock into 10 mM Tris/0.25 mM EDTA (pH 7.5) which contained variable concentrations of added NaCl. The final core particle concentration ranged from $A_{260} \simeq 0.25$ to $A_{260} \simeq 50$, and the final NaCl concentration ranged from 0 to 1.9 M. Glycerol (2–5%) was present in the solutions to aid in loading on gels. At various times after a jump, aliquots of the solution to be examined were loaded onto a native gel and were run under an electrical potential of 10–30

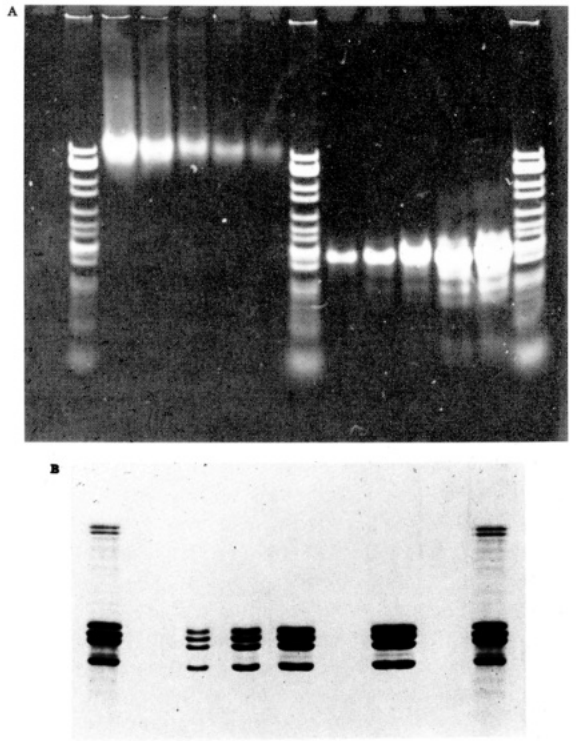

FIGURE 1: Characterization of nucleosome core particles, core particle DNA, and histones by gel electrophoresis. (A) A core particle solution was made at A_{260} = 4.7 (470 ng/μL) in 10 mM Tris, 0.25 mM EDTA, and 10% glycerol (pH 7.5). No dye was present. A Hamilton syringe was used to load amounts of 940, 470, 240, 90, or 50 ng in wells 2–6, respectively, of a native gel. Purified core particle DNA was dissolved at 235 ng/μL in 10 mM Tris, 0.25 mM EDTA, and 0.00125% bromophenol blue/xylene cyanol. This was loaded in amounts of 25, 50, 120, 240, or 470 ng in wells 8–12, respectively, of the same gel. Markers in lanes 1, 7, and 13 are *Cfo*I-cut pBR322, in the same buffer as the core particle DNA. From the top, the marker sizes are 393, (348 + 337 + 332), 270, 259, 206, 190, 174, (153 + 152 + 151), 141, (132 + 131), 109, 103, 100, 93, 83, and (75 + 67 + 62 + 60); below these bands, there is an unresolved smear of smaller DNA fragments. (B) Purified core particle histones, at 3.8 μg/μL in 60 mM Tris (pH 9), 2% SDS, 5% β-mercaptoethanol, 10% glycerol, and 0.001% bromophenol blue, were heated to 100 °C for ~5 min and then placed on ice. Amounts of 2, 4, 8, and 16 μg, respectively, were loaded in wells 2–5 of a Laemmli-type protein gel. Calf thymus histones (Worthington) were prepared similarly and were loaded in 20-μg amounts in wells 1 and 6. Staining was with Coomassie blue.

V/cm. The power source was turned on between each loading so that each aliquot would sit in a sample well for the minimum possible time before entering the gel. Multimer bands from some native gels were electroeluted directly onto Laemmli-type SDS–polyacrylamide gels for histone analysis. In such cases, 6-mm-thick 15% Laemmli gels were used, with 6% stackers [30:0.9 acrylamide:bis(acrylamide) ratio].

Results and Discussion

Characterization of Our Core Particles

We have taken particular care to prepare and define the nucleosomal core particles to be used in this study. We have characterized the core particle preparations by three different types of gel electrophoresis, sedimentation velocity, and circular dichroism. The core particles were found to be highly homogeneous, containing only the inner core histones and 144 ± 2 bp of DNA.

Gel Electrophoresis. Figure 1A shows *intact* core particles prepared by method I and run on a native gel at successively lower loadings (lanes 2–6). There are no nucleoprotein bands other than the main core particle band. In the limit of a low loading, the core particle band is quite narrow, indicating that the length of the core particle DNA is highly homogeneous. Even at high loadings, there is *no trace* of free DNA in this core particle preparation. Occasionally, core particle preparations do contain small traces of free DNA. However, quantitation with DNA standards indicates that the free DNA content of our core particle preparations is never more than 1–2%. We have noted that when bromophenol blue is included in the sample buffer, some nucleosome dissociation is induced (not shown). Therefore, a "native gel" assay *must* be done without dye in the sample buffer.

The gel shown in Figure 1A also displays successively higher loadings of the DNA that was purified from (method II) core particles (lanes 8–12). DNA from core particles prepared by method I looks essentially the same as this. On this native gel, the DNA runs in double-stranded form (Kovacic, 1976; Kovacic & van Holde, 1977). The center of the DNA size distribution lies at ~144 bp, and the breadth of the distribution is about ±2 bp. At the highest loads (lanes 11 and 12), traces of DNA are observed at regular intervals below the main 144 bp band. We believe that these additional bands were generated by internal cutting by micrococcal nuclease.

We have also examined core particle DNA on "single-stranded DNA" gels (not shown) to test for *periodic nicking*. Approximately 96% of the DNA from (method II) core particles ran homogeneously between 130 and 150 nt. At high loadings, approximately 4% of the DNA was found in bands around 125, 115, and 85 nt; presumably, these DNA species were generated by micrococcal nuclease during preparation of the core particles. Less of this periodic nicking was seen for the DNA of core particles prepared by method I. (Method I involves less exposure to micrococcal nuclease than does method II.) We note, however, that such gels will not detect low levels of *random* nicking, which will yield only a low-intensity smear on the gel.

Figure 1B shows the histones of core particles prepared by method II. Total protein from core particles was prepared and run on a 15% Laemmli-type gel, and the gel was stained with Coomassie blue. Lanes 2–5 show successively higher loadings of sample, while lanes 1 and 6 show a standard of calf thymus histones. The staining intensities of the four core histones from the core particle sample are about equal, indicating their molar ratios are approximately equal. We have noted that the cation-exchange resin used during "stripping" has been found to remove trace amounts (<1%) of the core histones from chromatin (Yager & van Holde, 1984).

At the highest loading on the protein gel (16 μg of material), we see no contamination by H1 or H5, and very little by non-histone proteins. In Coomassie staining of the highest loaded lane on this gel, the detection limit for residual H1, H5, or a non-histone protein is ~1% of a core histone band. One should not take this analysis to prove the *complete absence* of contaminant proteins. When the gel of Figure 1B is restained with silver, we are able to see some H1 and H5 and also about 10 other minor bands (not shown). However, densimetric analysis indicates that the sum of the contaminating bands represented less than 1% of the total core particle protein.

Sedimentation Velocity and Circular Dichroism. Our conclusions about particle homogeneity are supported by analytical centrifuge data for (method I) core particles. When a sedimentation velocity run in 10 mM Tris, 0.25 mM EDTA, and 0.5% sucrose, pH 7.5, was analyzed by the integral-distribution-of-S method (van Holde & Weischet, 1978), we found virtually all material in a single homogeneous boundary with a sedimentation coefficient $s_{20,w}$ = 10.7 ± 0.15 S. Direct

examination of the scans reveals a very small amount of a 5–6S component. By extrapolating the observed percentage of this component to zero sedimentation time, we estimate from the analytical centrifugation data that there is <2% free DNA in the sample.

A circular dichroism spectrum is a sensitive indicator of core particle integrity (Sahasrabuddhe & van Holde, 1974; Cowman & Fasman, 1978; Weischet et al., 1978). The CD spectrum of (method II) core particles displayed the following features in 10 mM Tris/0.25 mM EDTA, pH 7.5: (i) a small negative peak at ~295 nm; (ii) a sharp maximum at ~282.5 nm, with ellipticity of 1400 ± 200 deg cm^2/mol; (iii) a shoulder at ~275 nm; (iv) a zero crossover point at ~270 nm. These features are diagnostic for compact core particles without linker DNA (Cowman & Fasman, 1978). We conclude from all of the above studies that the core particles used in these experiments were homogeneous, not appreciably contaminated by free DNA, and of native conformation. It should be noted, however, that occasional preparations did not quite meet these standards. In one instance, a trace of dinucleosomal contamination was observed, and in another, about 5% of the DNA had been cut to sizes smaller than 145 bp. These preparations were used only in some of the high-salt studies, and we believe that the minor contamination should not significantly affect the results or conclusions.

Stability of Core Particles during Storage. Yager and van Holde (1984) report that, when nucleosomes are stored for prolonged periods at a concentration of $A_{260} \simeq 1$, as little as 10–25 mM NaCl can induce the release of a small fraction of the DNA. Also, it is well-known that the histones within core particles are sensitive to degradation. Although our particles were stored in the absence of added salt, we felt it important to examine the stability of our stock solutions.

A stock solution of (method II) core particles was stored at a concentration $A_{260} \simeq 20$–50 in 10 mM Tris/0.25 mM EDTA (pH 7.5) at $T = 0$ °C. After 6 weeks, the particle integrity was examined by three types of electrophoresis. We observed no trace of histone degradation on Laemmli-type protein gels, highly overloaded and stained with Coomassie blue. Core particles migrated as a single narrow band on native gels, although several percent more free DNA was observed than was originally present. When DNA was purified from the core particles and examined on a native gel, we observed ~94% of the DNA migrating at 144 ± 3 bp, and the remaining ~6% distributed in discrete bands at ~100, ~85, and ~40–50 bp. We concluded that after 6 weeks storage at 0 °C, there had been no histone degradation and only a slight degradation of the DNA in our core particles. Since no preparations over 6 weeks old were ever used, and most experiments utilized preparations no more than 3 weeks old, we do not consider this slight degradation to be a serious problem.

Release of DNA from Nucleosomes

To determine the range of conditions under which DNA could be released from well-defined core particles, and to search for intermediates in the DNA release process, we used preparations that showed little or no (≤2%) free DNA in low-salt buffer (Figure 1A). Three techniques were used to monitor the release of DNA or histones: (1) analytical centrifugation, in which free DNA is indicated by a 5–6S UV-absorbing boundary; (2) electrophoresis on native gels, in which free DNA is indicated by a band with electrophoretic mobility corresponding to DNA of 145 bp length; (3) analytical gel filtration chromatography, in which histone–DNA complexes can be resolved from free histones. As we will demonstrate

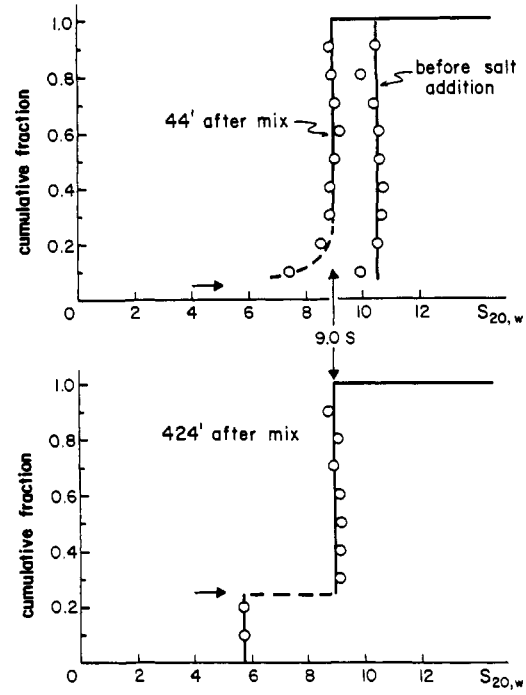

FIGURE 2: Dissociation of nucleosomes as monitored by sedimentation coefficient distribution. The method of van Holde and Weischet (1978) was used to analyze the integral distribution of sedimentation coefficients of core particles before and after a salt jump to 0.75 M NaCl. In the top panel, the data to the right correspond to native core particles in low-salt buffer (10 mM Tris/0.25 mM EDTA, pH 7.5). The curve to the left (top panel) shows the distribution observed after an aliquot of a concentrated nucleosome stock was diluted into low-salt buffer which had been made 0.75 M in NaCl and incubated for 44 min at 20 °C. In the lower panel are shown data from a sedimentation experiment begun 424 min after mixing. At 44 min, only partial dissociation seems to have occurred (arrow), whereas by 424 min about 25% dissociation is observed.

below, the character of the nucleosome's response to salt is different below and above about 0.75 M. Therefore, we shall describe results in these two regions of salt concentration separately.

DNA Release in Moderate (0.1–0.75 M) [NaCl]. (A) Analytical Ultracentrifugation. Core particles were jumped to moderate concentrations of NaCl by diluting a low-salt stock into 10 mM Tris/0.25 mM EDTA (pH 7.5) containing 0, 0.1, 0.3, 0.5, or 0.75 M added NaCl. These core particle solutions were maintained at 20.5 ± 1.5 °C. The final concentration of material gave a total absorbance $A^{1cm}_{265nm} \simeq 0.3$. A 30-mm ultracentrifuge cell was used to give an effective absorbance of 0.9. At various times after the dilution into elevated NaCl concentrations, aliquots of each of the solutions were examined in the analytical ultracentrifuge. With the most rapid handling, an aliquot of core particles could be placed in a cell and brought to speed in about 40 min. Therefore, the earliest time points we were able to obtain by this method were taken ~40 min after the dilution into elevated NaCl.

Figure 2 presents sedimentation data obtained before a salt jump to 0.75 M NaCl and in runs made approximately 44 and 424 min after the jump. The data have been analyzed by the method of van Holde and Weischet (1978), which effectively removes diffusion broadening of the boundaries. The results of such experiments reveal two phenomena associated with the salt jump: (1) There is production of a homogeneous component sedimenting at 5–6 S. We have demonstrated in an earlier study (Yager & van Holde, 1984) that this boundary corresponds to free DNA. Thus, a certain fraction of the nucleosomes have undergone dissociation. (2) Following a salt

jump, there is a decrease in the sedimentation coefficient of the remaining major component. This phenomenon has been observed by many workers [i.e., see Stacks and Schumaker (1979), Wilhelm and Wilhelm (1980), Russev et al. (1980), Eisenberg and Felsenfeld (1981), Ausio et al. (1984), and Yager and van Holde (1984)] and will not be considered further here; a detailed analysis will be the subject of a future publication. We return to a description of the dissociation process.

In the earlier studies (Yager & van Holde, 1984), using nucleosomes containing longer DNA, this dissociation was observed to be a slow process. With core particles, we find similar evidence for slow kinetics; in each of the salt jump experiments, the first sedimentation experiment (~40 min after the jump) shows less dissociated DNA than in all later experiments (see Figure 2). However, the dissociation process with core particles is faster than observed with the long DNA nucleosomes used earlier. It is in fact too fast to allow us to analyze the kinetics by sedimentation experiments.

We find that the equilibrium value for the percentage of slow boundary increases with increasing salt concentration. This has been noted also by others (Stacks & Schumaker, 1979; Vassilev et al., 1981; Cotton & Hamkalo, 1981; Ausio et al., 1984; Yager & van Holde, 1984). Over the 0–0.75 M NaCl range, our data can be described approximately by a linear equation of the form % free DNA = 2.5% + (29.1%)X, where X = molarity of NaCl. This relationship is true when the core particle concentration is held at 140 nM ($A^{1cm}_{265nm} \simeq 0.3$) and the temperature is held at 20.5 ± 1.5 °C (data not shown). The results are in general agreement with those reported by Ausio et al. (1984).

In order to investigate the concentration dependence of the dissociation, additional experiments were performed in 0.5 M NaCl at core particle concentrations corresponding to $A^{1cm}_{265nm} \simeq 1$, 3, 5, and 10. We observe only a trace of dissociation at $A_{265} = 1$ and no dissociation at the higher concentrations. In this respect, our results differ from those of Ausio et al. (1984), who report residual dissociation even at high concentrations. We believe that this may have resulted from contamination of their starting sample with free DNA, which would also explain their observation of somewhat greater dissociation at very low salt. Our results are in qualitative accord with expectations from the mass action law.

A major point that emerges from the sedimentation analysis presented here is that there is no evidence for any intermediates in the dissociation process. The remaining nucleosomes exhibit a sharp sedimentation coefficient distribution. There is no indication in any of our experiments, even at the highest concentrations, for components sedimenting more rapidly than core particles, as would be expected if the dissociation involved a disproportionation mechanism involving histone octamer–core particle complexes (Stein, 1979; Ausio et al., 1984).

The sharp break between the fast and slow boundaries (Figure 2) would seem to argue for the absence of intermediates in which a portion of the histones had been lost. However, care is required, for rapidly equilibrating systems can sometimes produce sharp reaction boundaries (Cann, 1970), and the van Holde–Weischet theory has not been extended to rapidly equilibrating systems. For these reasons, we have turned to other techniques to search for intermediates.

(*B*) *Analytical Gel Filtration.* We have conducted a set of experiments in which core particle preparations ($A_{260} = 5.2$) were loaded on Bio-Gel P-100 columns, with or without 0.6 M NaCl in the column buffer. On this column, core particles, free DNA, and even core particles which had suffered some histone loss will elute in the void volume. However, if there is *preferential* loss of some histones (i.e., H2A and H2B), these histones should be left behind, and the void volume material should exhibit the histone loss by exhibiting a histone stoichiometry deficient in these histones. The void volume material was checked in three ways for partial loss of histones.

(*1*) *Native Gels.* A low-salt sample was loaded directly onto a P-100 column equilibrated in 0.6 M NaCl. The void volume material was collected and examined on a native gel (Figure 3A, bottom three traces). We observed the appearance of some free DNA (high-mobility band in each trace), indicating that a fraction of the core particles had dissociated, as expected. The major component, however, ran homogeneously with exactly the same electrophoretic mobility as intact core particles (compare with top two traces). In a similar gel electrophoresis assay for core particles that were partially dissociated by ethidium bromide, a particle containing only a hexamer of core histones could be easily resolved (McMurray & van Holde, 1986). We see *no* evidence for any such intermediate here.

(*2*) *Protein Gels.* After a 0.2–24-h preincubation in 0.6 M NaCl, core particles were loaded onto a P-100 column in 0.6 M NaCl. This histone composition of the void volume material was determined on Laemmli gels. After any length of preincubation, the void volume material always retained an H2A + H2B histone percentage of 50 ± 2% (data not shown). This constitutes a second argument that a subset of histones are not preferentially lost from the core particles in 0.6 M NaCl.

(*3*) *Absorbance Ratio.* Figure 3B provides a third line of evidence that no subset of histones is lost preferentially in 0.6 M NaCl. This figure shows the ratio of absorbances at 220 nm and at 260 nm for material eluting in the P-100 column's void volume. The upper dotted line shows the A_{220}/A_{260} ratio we expect for intact core particles. The middle and bottom dotted lines show the A_{220}/A_{260} ratio we expect for particles that are depleted of one or two histone dimers, respectively. The spectral ratio that we observe over a braod range of particle concentrations is most consistent with a model in which there is no preferential loss of some histone types from the void volume material. (The line-of-best-fit does fall slightly below the upper dotted line in Figure 3B. This most likely reflects the complete dissociation of a fraction of the core particles, as is shown in the bottom three traces of Figure 3A.)

Taken together, the data described above demonstrate that the dissociation of core particles in the salt concentration range between 0.1 and 0.75 M NaCl can be adequately described as a two-state process. Intermediates, if they exist, must be transient, for they are undetectable by any means we have used.

DNA Release in High (>0.75 M) [NaCl]. Earlier studies have shown that, for *long chromatin fragments*, there is a selective loss of equimolar amounts of H2A and H2B as the salt concentration is raised above ~0.8 M (LaRue & Pallotta, 1976; Wilhelm et al., 1978; Burton et al., 1978, 1979; Jorcano & Ruiz-Carrillo, 1979; Oohara & Wada, 1987). This argues that at high salt the release of histones may no longer be highly cooperative. We wanted to reexamine this phenomenon with relatively homogeneous preparations of core particles.

(*A*) *Analytical Ultracentrifugation.* Two questions are accessible with ultracentrifugation methods. (1) Are nucleoprotein species generated that are intermediate in size between free DNA and intact core particles? (2) Are high molecular weight intermediates generated, consisting of core particles with additional histones bound?

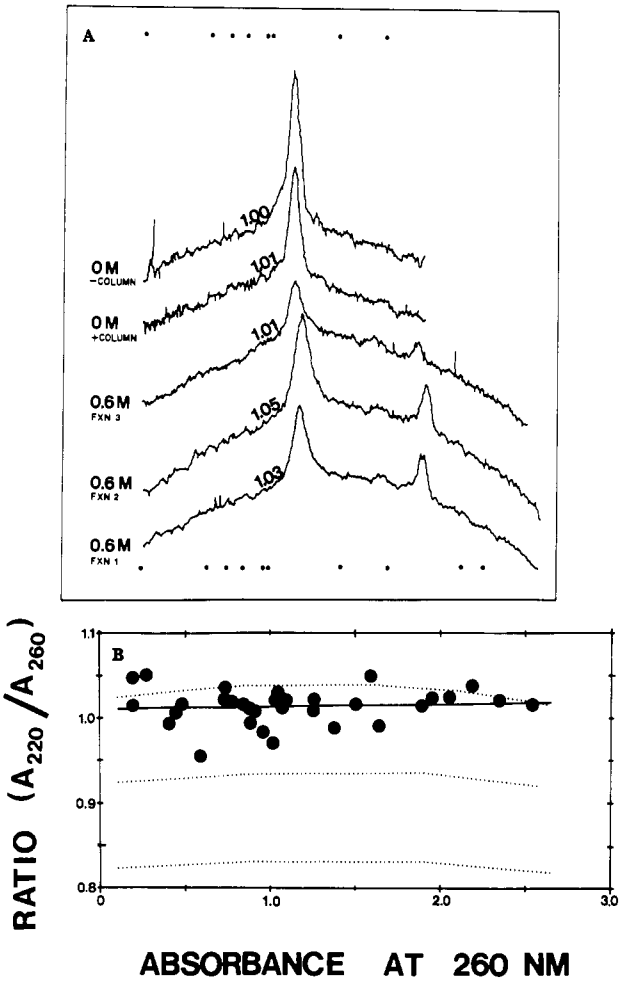

FIGURE 3: Test for partial loss of histones from core particles, after a jump to 0.6 M NaCl. (A) Electrophetic analysis after gel filtration; 100 μg of core particles in 100 μL of 10 mM Tris/1 mM EDTA, pH 7.2 ($A_{260} \simeq 10$), was loaded onto a 6.8 cm × 0.8 cm Bio-Gel P-100 column in 5 mM Tris (pH 7.8) ± 0.6 M NaCl. Fractions from either column were made 5% in glycerol (no dyes) and were run on native gels. Calibration of gels was with a BSP1286 digest of pBR322 DNA. Trace 1 (top trace): core particles in low salt, which never contacted a column. Trace 2: void volume material from the low-salt column. Traces 3–5: material from successive void volume fractions of the 0.6 M NaCl column. Traces 1 and 2 were from a single gel (calibration = upper dots); no free DNA could be seen. Traces 3–5 were from a second gel (calibration = lower dots); some free DNA is apparent (right-hand side of each trace). The relative mobilities of the core particle bands are indicated. (B) Spectral ratio (A_{220}/A_{260}) after gel filtration. Core particles were diluted from a low-salt stock into 5 mM Tris, pH 7.5. They were then loaded immediately onto a Bio-Gel P-100 column equilibrated in 0.6 M NaCl/5 mM Tris (pH 7.5). EDTA was left out of the loading buffer and the column buffer, so that the buffers would have a low optical absorbance at λ = 220 nm. The temperature was 22 ± 1 °C. Fractions from the column were collected, and the two or three most concentrated ones (from the void volume) were analyzed for the A_{220}/A_{260} ratio on a Hewlett-Packard 8450A spectrophotometer. No fractions were used that fell outside the tested range of Beer's law validity ($A_{260} \simeq 0.1$–2.6). The results of 17 independent column runs are indicated by black circles. The horizontal back line is the linear least-squares fit through the points: it indicates an A_{220}/A_{260} ratio of 1.01 and no significant concentration dependence (slope = +0.0015). The upper dotted line indicates the ratio expected for an intact core particle in 0.6 M NaCl. The middle and lower dotted lines indicate the ratios expected for core particles missing one-fourth or half, respectively, of the normal complement of histones. The dotted lines were obtained by spectrophotometric examination of core particles and DNA in 0.6 M NaCl/5 mM Tris (pH 7.5).

To answer these questions unambiguously, we performed analytical ultracentrifugation of core particles in 1.0 and 1.5 M NaCl, at concentrations of 0.3, 1, 3, 5, and 10 A_{265} units.

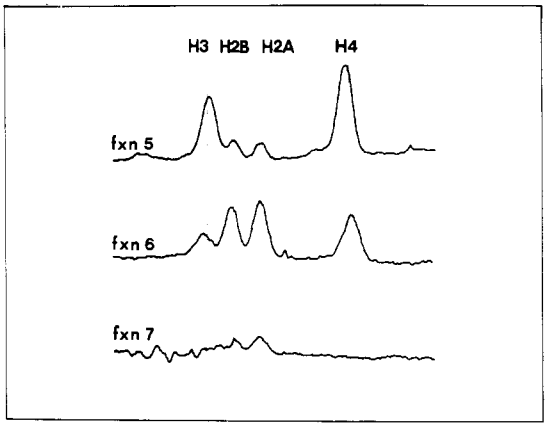

FIGURE 4: Dissociation of H2A and H2B from core particles in 1.5 M NaCl. Core particles were diluted from a low-salt stock to a concentration $A_{260} \simeq 5.2$ in 1.5 M NaCl/5 mM Tris (pH 7.5). The temperature was 22 ± 1 °C. After an incubation period of 24 h, 100 μL of the material was loaded onto a 6.8 cm × 0.8 cm Bio-Gel P-100 column in 1.5 M NaCl/5 mM Tris (pH 7.5). Fractions (150 ± 20 μL in volume) were collected, heated to 95 °C in the presence of SDS and β-mercaptoethanol, and loaded onto a Laemmli-type protein gel. The protein gel was stained with Coomassie blue and photographed. The film negative was scanned on a laser densitometer (Biomed Instruments). From top to bottom in this panel, we show the histone composition of fractions 5, 6, and 7 from the P-100 column. The void volume falls at fractions 4–5.

At either NaCl concentration, only a homogeneous boundary corresponding to free DNA is observed when the nucleoprotein concentration is A_{265} = 0.3. However, as the nucleoprotein concentration is raised, a heterogeneous boundary is observed, sedimenting between 5 S and 10 S.

The series of experiments in 1.5 M NaCl was analyzed in more detail, using the method of van Holde and Weischet (1978). The following results were obtained. At A_{265} = 1.0, only a homogeneous 5.3S boundary is observed. At A_{265} = 5.0, about 30% of the material sediments at 7.0 S, with the remainder of the boundary trailing toward ~5 S. At A_{265} = 10, approximately half of the material sediments at about 7.2 S. There is still trailing of the material toward ~5 S, but the amount of this is decreased as compared to the sample at A_{265} = 5. Thus, there is a heterogeneous population of partially dissociated particles that is driven toward a more associated state by an increase in nucleoprotein concentration. Even at A_{265} = 10, however, the upper bound of the distributon of s falls significantly below 10 S. This indicates that *no* intact core particles are present at 1.5 M NaCl. Again, as in the experiments at low salt, we never see any trace of material sedimenting faster than 10.7 S.

(*B*) *Analytical Gel Filtration Chromatography.* We hypothesized that the heterogeneous nucleoprotein boundary observed in the ultracentrifuge in 1.0–1.5 M NaCl consisted of core particles that were partially depleted of histones. In an attempt to test this hypothesis directly, we conducted a number of gel filtration experiments at an NaCl concentration of 1.5 M. Core particles were diluted from a low-salt stock to 1.5 M NaCl and a particle concentration A_{260} = 5.2. They were incubated for 24 h and were then loaded onto a P-100 column in 1.5 M NaCl ($T \simeq 22$ °C). Figure 4 shows that the material eluting in the void volume (fraction 5; $A_{260} \simeq$ 2.5, M_r >100 000) is depleted of H2A and H2B to an extent of ~80%. In contrast, the material in fraction 6 (significantly after the void volume) is enriched in H2A and H2B. Thus, in contrast to the behavior in moderate (0.1–0.75 M) salt, there appears to be a preferential release of H2A and H2B from core particles in high (1.5 M) salt. Interestingly, the H2A

and H2B in fraction 6 do *not* elute from the column in the manner expected for free histone monomers or dimers; such material should elute around fractions 10–12 (column calibration data not shown). Thus, the H2A and H2B may have enough residual affinity either for free DNA or for DNA–$(H3{\cdot}H4)_2$ complexes to be carried along with the void volume material part of the time. Alternatively, it is possible that there is some aggregation of H2A·H2B dimers under these conditions.

We have also conducted a series of column experiments designed to measure the time course of release of H2A, H2B from core particles in 1.5 M NaCl. We find that the release occurs within a time frame of minutes—not instantaneously, and not over hours (data not shown).

The major conclusion from the above experiments is that a heterogeneous population of nucleoprotein particles is generated by exposure to 1.0–1.5 M NaCl. In terms of association state, this population is bounded from below by free DNA and is bounded from above by particles sedimenting more slowly than intact core particles. The "average association state" of this population of particles seems to be dictated by the law of mass action. Thus, in high salt, histone release is *not* a highly cooperative process; rather, the nucleosomes dissociate in a *stepwise* fashion, reminiscent of that observed for long chromatin under similar salt conditions. We may hypothesize that the dissociation mechanism is fundamentally different. At ionic strengths below 0.75 M NaCl, dissociation may occur by a DNA sliding mechanism (Beard, 1978; van Holde & Yager, 1985). Sliding, while capable of rearranging long chromatin, will not lead to its dissociation. Thus, nucleosomes and long chromatin behave differently at low salt. On the other hand, above 0.75 M, both long chromatin and nucleosomes lose H2A and H2B. This will lead to the formation of intermediate particles and a rapid dissociation when nucleosomes are studied under these conditions.

It should also be noted that the absence of any components sedimenting more rapidly than core particles argues against any disproportionation model under these conditions.

Stability Diagram for the Nucleosome

We are now in a position to consider the problem of nucleosome stability in more general terms. Figure 5 proposes a stability diagram for the nucleosome. Stability at a given temperature (in this case, 20 °C) is considered to be a function of two independent variables: nucleosome concentration and salt concentration. From studying our own data and data from the literature, we conclude that there is evidence for five major regions in the stability diagram.

Region 1 describes the native nucleosomal state in which two copies each of H2A, H2B, H3, and H4 are stably bound to a piece of DNA, to give the well-characterized compact particle. This state exists under low to moderate salt conditions (ionic strength = 0.002–0.75 M), the upper limit depending upon nucleosome concentration.

Region 1E describes the "low-salt expanded" nucleosomal state, which is reached from the native state (region 1) by lowering the ionic strength to ≤1 mM (Wu et al., 1979; Burch & Martinson, 1980; Libertini & Small, 1982; Uberbacher et al., 1983). The expansion of the nucleosome most likely results from increased electrostatic repulsion between phosphates in the DNA backbone. The 1E region of the stability diagram may actually consist of two or more subregions (Gordon et al., 1978, 1979; Burch & Martinson, 1980; Harrington, 1981).

Region 2 describes a situation in which the native nucleosome is in equilibrium with free DNA and free histones. There are no *detectable* intermediates or side products of the dissociation process in this region of the stability diagram. This region is entered at moderate nucleosome concentrations (A_{260} ≃ 0.3) and moderate salt concentrations (0.1–0.75 M NaCl).

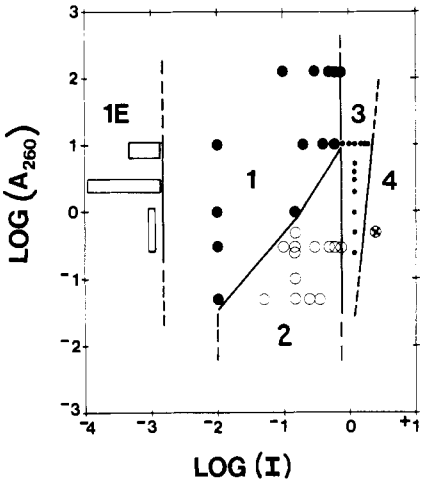

FIGURE 5: Stability diagram for the nucleosome. The following regions of stability are indicated in this diagram: (1) the "native" state of the nucleosome; (1E) the "low-salt expanded" state of the nucleosome; (2) the highly cooperative equilibrium between the "native" nucleosome, DNA, and histones; (3) the relatively noncooperative equilibrium between the "native" nucleosome, DNA, and histones; (4) the fully dissociated state of DNA + histones. We base this diagram on the following experimental data. Region 1 (large closed circles): "native" gels, this paper; neutron scattering, unpublished observations; also data of Cotton and Hamkalo (1981). Region 1E (open rectangles): data of Libertini and Small (1982) and Uberbacher et al. (1983). Region 2 (open circles): analytical centrifugation, gel filtration, and "native" gels, this paper; also data of Cotton and Hamkalo (1981). Boundary between region 1 and region 2: data of Beard (1978) and Louters and Chalkley (1984). Region 3 (small solid circles): gel filtration and analytical centrifugation, this paper. Region 4 (cross-in-circle): data of Stein (1979).

Region 3 describes a situation in which the native nucleosome is in equilibrium with free DNA, free histones, and (H2A,H2B)-depleted particles. This region is entered at high salt concentrations (0.8–1.5 M NaCl).

Region 4 describes a situation in which the DNA and the histones are completely separate and do not interact. It is reached only at very high salt concentrations [>1.5 M; see, e.g., Stein (1979)].

The distinction between a "stable native nucleosome" and a "dissociating nucleosome" may sometimes be vague or ill-defined. For example, some small amount of nucleosome dissociation must persist even at very high nucleosome concentrations and low salt concentrations, although equilibrium may be attained very slowly under conditions where sliding is strongly hindered. Similarly, there is evidence [i.e., see Louters and Chalkley (1984)] that H2A·H2B dimer *exchange* may occur under conditions where we can detect no significant fraction of nucleosomes which have lost these dimers.

Reversibility of Transition between Different Regions of the Stability Diagram

The question arises as to whether nucleosomes can pass reversibly between the different "regions of stability" of Figure 5.

The 1 ⇄ 1E Transition. One study suggests that the transition between the native and low-salt nucleosomal structures is reversible, although it may involve multiple steps (Eshaghpour et al., 1980). This study may be faulted, however, because it uses nucleosomes modified by fluorescent adducts at the –SH moieties of the H3 Cys-110 residues. More recently, it has been observed that unmodified core

particles taken to *very* low ionic strength cannot be completely returned to state I by increasing the salt (Libertini & Small, 1987).

The 1 ⇄ 2 Transition. Yager and van Holde (1984) and Ausio et al. (1984) have described experiments which argue for the reversibility of this DNA release reaction. Reversibility may be hampered somewhat by a slow and nonspecific aggregation of the histones.

The 1 ⇄ 3 Transition. To our knowledge, no direct studies have yet been performed on the reversibility of this transition in core particles. However, nucleosomes can be reversibly passed between regions 1 and 4 (see below), passing through region 3. This strongly implies that the 1 → 3 transition must be reversible, although the qualifications given below may well apply.

The 1 ⇄ 4 Transition. It is well-known that nucleosomes can be reconstituted in high yield by mixing histones and DNA in high salt and then dialyzing slowly to low salt [see, e.g., see Tatchell and van Holde (1977, 1979)]. Thus, the 1 ⇄ 4 transition is highly reversible when the salt removal step is done *slowly*. However, a complication arises when the salt is removed *rapidly* by a "jump-dilution" technique. In this case, it has been reported that a 13S particle is generated which may be a reassociation intermediate [Figure 8F of Stein (1979)]. We know from the experiments described above that such particles are not *stably* present at any salt concentration. Therefore, in order to examine conditions under which they might be formed, we performed the following set of experiments.

In an experiment analogous to a jump-dilution, a high-salt solution of core particles was loaded onto a native gel. As the nucleoprotein material enters the gel, the salt is rapidly diluted away, and kinetic intermediates in reassociation may become trapped. The results are shown in Figure 6A, for core particle samples at an initial concentration of A_{260} = 5.2. When loading is in 1.0 M NaCl, virtually no species other than a band of intact core particles is seen on the gel. In contrast, gels loaded at 1.2, 1.5, and 1.8 M NaCl demonstrate both free DNA and a *series* of ethidium-staining bands of low electrophoretic mobilities.

The low-mobility bands of Figure 6A might be multimers of the core particle, multimers of some histone-depleted particle, or core particles to which increasing numbers of histone octamers have bound. The low-mobility bands do in fact behave electrophoretically like multimers of some precursor. This is demonstrated by assuming the bands have integral multiples of a monomer molecular weight and then plotting the logarithm of the relative multimer weights versus the bands' electrophoretic mobilities (Figure 6B). A linear plot is obtained which, by extrapolation, yields a "monomer" electrophoretic mobility close to that of an intact core particle. This result strongly suggests that the low-mobility bands we observe are multimers either of a core particle or of some structure derived from it by partial histone dissociation. The fact that a linear relationship is observed suggests a constant charge/mass ratio, arguing against the proposition that these components are simply core particles with added histones. If the latter were the case, we would expect a varying charge/mass ratio, and thus a nonlinear behavior in the plot shown in Figure 6B.

To better understand the composition of the low-mobility bands, we have incubated a core particle sample in 1.5 M NaCl and then jump-diluted it onto a gel as described above. The resulting "monomer", "dimer", and "trimer" bands were cut out and analyzed for histone contents on a Laemmli gel. We find, for each of these bands, equimolar amounts of each of the four histones (data not shown). This result, in combination with our interpretation of Figure 6B, indicates that a jump-dilution experiment will generate *multimers of core particles*.

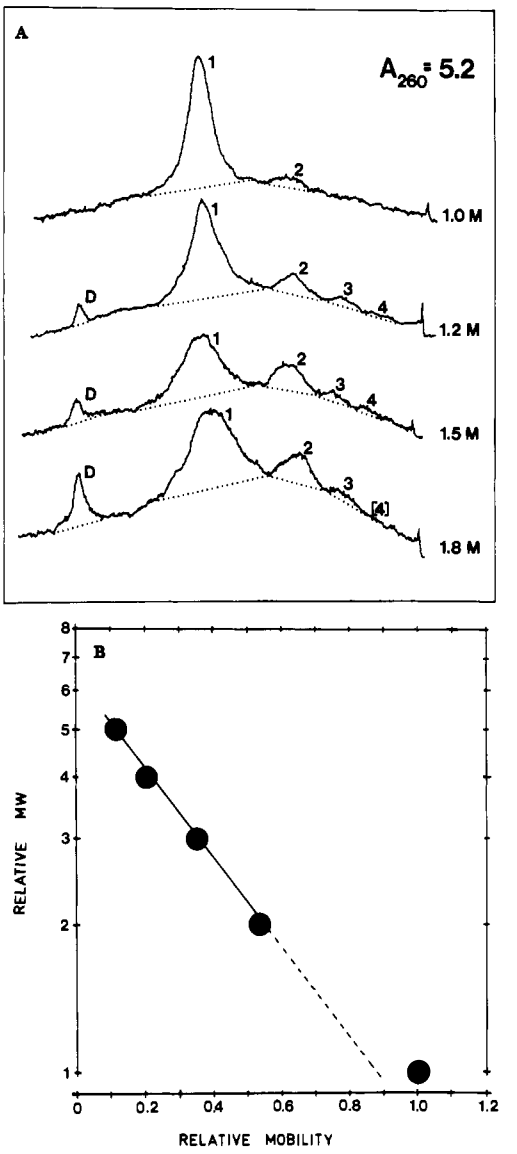

FIGURE 6: Generation of nucleosome "multimers". Core particles were diluted from a low-salt stock to conditions described below. Aliquots were run immediately on native gels. The gels were stained with ethidium bromide and photographed under long-wavelength UV light. The film negatives were scanned with a laser densitometer (Biomed Instruments). (A) Dependence on NaCl concentration (core particles at $A_{260} \simeq 5.2$). The jump was made to a core particle concentration $A_{260} \simeq 5.2$ and an NaCl concentration of 1.0, 1.2, 1.5, or 1.8 M. (B) Electrophoretic mobilities. Some of the above gels were used to determine relative mobilities of the apparent multimer bands. We assumed these bands to be integral molecular weight multiples of a monomeric precursor. The assumed relative molecular weight was plotted versus the relative mobility, on semilog paper. A relative mobility of 1.0 was assigned to the intact core particle band. Extrapolation of the "multimer series" leads to an estimated mobility of 0.9 for the (hypothetical) monomeric precursor of the "multimer" ladder.

Finally, we wished to explore the idea that multimer generation is a transient phenomenon unique to the jump-dilution technique. Aliquots of core particle samples at A_{260} = 10.4 in 1.9, 1.8, 1.5, or 1.2 M NaCl buffer were loaded onto gels, as above; each generated a series of multimer bands. The remainder of each sample was then diluted 1:1 with low-salt buffer, to bring the salt concentration down to 0.95, 0.9, 0.75, or 0.6 M, respectively. The samples were incubated for varying

lengths of time at the lower salt concentrations and then were again subjected to jump-dilution by loading onto gels. We found decreasing amounts of the multimer bands the longer the incubation was carried out (data not shown). This suggests that, during a slow salt dialysis reconstitution (4 → 1 transition in Figure 5), the potential to form multimers is slowly lost as the salt concentration is decreased.

The above experiments allow us to form a hypothesis for the structure of the multimers. We propose that, in a jump-dilution experiment, histone octamers rapidly reassociate with DNA in a way that is out of register. This would allow core particle multimers to form via DNA bridges between histone octamers. Such multimers should be, at best, metastable. If maintained at intermediate ionic strengths, they would slowly rearrange, to be replaced by more stable monomeric particles with the DNA in proper register on the histone core. This we propose to occur via the process of core sliding (Beard, 1978; van Holde & Yager, 1985).

SUMMARY

This paper has focused on the reaction by which DNA is released from nucleosome core particles. We show that this reaction can proceed by either of two mechanisms. The first mechanism, occurring at NaCl concentrations <0.75 M, involves a highly cooperative release of the histones from the DNA, so that no kinetic intermediates are never observed. It seems likely that DNA sliding is involved in this process. The second mechanism, occurring at NaCl concentrations ≥0.75 M, probably involves an initial loss of H2A and H2B, triggering a rapid dissociation. The dissociation which occurs at moderate salt is readily reversible. Dissociation in the high-salt range is reversible if the reassociation is conducted slowly (as by gradient dialysis). However, rapid dilution to low salt leads to the formation of metastable multimers of the core particle.

The experiments have led us to present a stability diagram for the core particle which is defined in terms of the two independent variables of core particle concentration and salt concentration. Five separate regions of stability are demarcated, between which reversible transition can occur.

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Chicken Globin Gene Transcription Is Cell Lineage Specific during the Time of the Switch†

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ABSTRACT: Posttranscriptional silencing of embryonic globin gene expression occurs during hemoglobin switching in chickens [Landes, G. M., Villeponteau, B., Pribyl, T. M., & Martinson, H. G. (1982) *J. Biol. Chem. 257*, 11008–11014]. Here we use Percoll density gradients to fractionate the red blood cells of 5–9-day embryos in order to determine the cellular source and the timing of this posttranscriptional process. By means of nuclear "run-on" transcription in vitro we show that it is within mature primitive cells that production of embryonic globin mRNA is terminated posttranscriptionally. In contrast, young definitive cells produce little (or no) embryonic globin mRNA because of regulation at the transcriptional level. Thus the lineage specificity of embryonic and adult globin gene expression is determined transcriptionally, and the post-transcriptional process described by Landes et al. is a property of the senescing primitive cells, not a mechanism operative in the hemoglobin switch. This conclusion is supported by [^{3}H]leucine incorporation experiments on Percoll-fractionated cells which reveal no posttranscriptional silencing of the embryonic genes during the early stages of the switch. In the course of our studies we have noticed a strong transcriptional pause near the second exon of the globin genes which is induced by 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) and which resembles a natural pause near that position.

During avian development there are, as in mammals, switches in the types of hemoglobins expressed. Prior to 6 days of incubation circulating erythroid cells in chicken embryos express exclusively embryonic hemoglobins while after the 12th day of development only adult hemoglobins are expressed (Bruns & Ingram, 1973). The molecular and cellular mechanisms governing these switches are difficult to address experimentally because a complete cellular change in erythroid lineage takes place simultaneously with the molecular hemoglobin switch. Erythroid cells of the primitive lineage arising from the blood islands of the area vasculosa are the first to enter circulation during the second day of incubation. These cells mature as a cohort and are the only recognizable erythroid cells in the embryo until the 5th day when cells of the definitive lineage begin entering circulation. As the circulation expands, the definitive erythroid cells rapidly outnumber the senescing primitive cells which become virtually undetectable by 16 days of development (Bruns & Ingram, 1973). The switch in hemoglobin expression and the switch in erythroid lineage are well correlated. Prior to 5 days of incubation only embryonic hemoglobins are expressed at a time when only primitive cells are found in circulation. Adult hemoglobins begin to appear during the 6th day of incubation simultaneously with the first appearance of definitive erythroid cells.

In mature primitive and definitive erythroid cells taken from embryos prior to (i.e., at 5 days) or following completion of (12–14 days) the hemoglobin switch, the choice among globins is made at the transcriptional level (Groudine et al., 1981; Landes et al., 1982). For such embryos, either before or after the switch, transcriptional specificity, translational output, and cell lineage are all correlated. However, during the actual period of switching (6–9 days), when both primitive and definitive cells are present simultaneously in the circulation, the rate of transcription of the embryonic genes remains disproportionately high, indicating the contribution of a posttranscriptional process that silences the embryonic genes (Landes et al., 1982). These and related data led to the suggestion that the embryonic globin genes are active in im-

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