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Physical Studies of Nucleosome Assembly†

L. Klevan, N. Dattagupta, M. Hogan, and D. M. Crothers*

ABSTRACT: Biochemical, spectroscopic, and hydrodynamic studies were performed on the reconstituted complex of 140 base pair DNA and the arginine-rich histone tetramer $(H3/H4)_2$. The histones bind to DNA in a 1:1 molar ratio to form a stable particle which orients in an electric field with a rotational correlation time of 6.3 μs and a limiting reduced dichroism of −0.74. The complex was modeled hydrodynamically as a cylinder of dimensions 450 × 80 × 80 Å containing approximately 1.5 superhelical turns. Addition of the lysine-rich histones to this complex causes a condensation of the structure and results in physical properties nearly identical with those of a native nucleosomal particle.

The arginine-rich histones H3 and H4 play a central role in packaging the DNA of eucaryotic chromosomes into an array of repeating subunits or nucleosomes (Oudet et al., 1975). Each nucleosome consists of a central core particle comprising 140 base pairs of DNA and two each of histones H2A, H2B, H3, and H4 (Kornberg, 1974; Olins et al., 1976) along with a spacer region of variable length (Spadafora et al., 1976; Lohr et al., 1977). Comparative studies of histone H4 from pea seedling and calf thymus have demonstrated a remarkable conservation of amino acid sequence for proteins derived from such diverse organisms, suggesting a strong structural-functional correlation for its interaction with DNA (DeLange et al., 1969). Likewise, histone H3 is unique in that it contains the amino acid cystine and may be involved in stabilizing the nucleosome during periods of genetic inactivity (DeLange & Smith, 1972; Camerini-Otero & Felsenfeld, 1977a).

Kornberg & Thomas (1974) reported the isolation of a stable arginine-rich histone tetramer which, when reconstituted with DNA and the lysine-rich histones reproduced the X-ray diffraction pattern of chromatin (Kornberg & Thomas, 1974). The stability of this protein complex was subsequently confirmed by equilibrium centrifugation (Roark et al., 1974; D'Anna & Isenberg, 1974a) and by studies on the reassociation of purified histone components (D'Anna & Isenberg, 1974b).

Physical studies on the histone tetramer indicate a highly asymmetric protein structure; the sedimentation coefficient $s_{20,w}$ is 2.81 and the ratio of the frictional coefficient to that of a sphere of the same volume (f/f_0) is approximately 1.6 (Roarck et al., 1976). Although the molecule contains a considerable amount of α helix, the proton NMR spectrum resembles that of a partially denatured protein (Moss et al., 1976). It has been suggested that the N-terminal cationic regions of these proteins are highly mobile (Lilley et al., 1976) and may be the primary sites of interaction with DNA (Sung & Dixon, 1970; Weintraub et al., 1976). The combination of a globular hydrophobic core and extended N-terminal hydrophilic regions could account for the observed hydrodynamic properties of the molecule (Roark et al., 1976).

Recently it has been demonstrated that the arginine-rich histones can supercoil DNA in the absence of the other histone components. Bina-Stein & Simpson (1977) observed that reconstitution of the histone tetramer with closed circular SV 40 DNA, followed by treatment with relaxing enzyme, could reproduce the superhelical density of virion DNA upon removal of the protein. The results of their study were consistent with the generation of approximately one negative superhelical turn per bound tetramer. Camerini-Otero & Felsenfeld (1977b) observed a significant amount of supercoiling upon reconstituting the tetramer with relaxed Col E1 DNA at a 0.5 g/g of protein/DNA ratio, although a nucleosome equivalent of protein was required to obtain a superhelical density identical with that of native DNA. No supercoiling was observed in either study upon reconstitution of the lysine-rich histones with DNA.

Moss et al. (1977) have obtained X-ray diffraction patterns very similar to that of chromatin from fibers of DNA reconstituted with the arginine-rich histones in a 1:1 weight ratio. It was suggested that these diffraction patterns might result from a structure in which one histone tetramer was bound to approximately 65 base pairs of DNA.

In this paper we report the stoichiometry and hydrodynamic properties of a reconstituted particle of the arginine-rich histones and 140 base pair DNA. In summary of our results, we

† From the Department of Chemistry, Yale University, New Haven, Connecticut 06520. *Received April 21, 1978.* This research was supported by Grant CA15583 from the National Cancer Institute.

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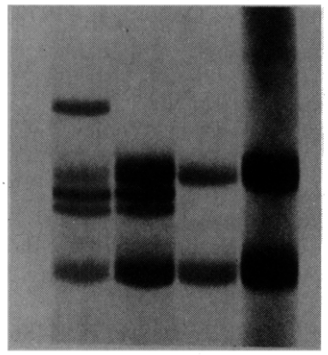

FIGURE 1: Fifteen percent acrylamide–2.5 M urea (pH 2.5) disc gels of histone proteins. From left to right: whole histones obtained by gel filtration on Sephadex G-50 SF. The protein bands from top to bottom represent histones H1, H3, H2B, H2A, and H4; histones precipitated by ammonium sulfate at 70% saturation; arginine-rich histones prepared by gel filtration of whole histones on Sephadex G-100 SF; arginine-rich histones prepared by gel filtration of ammonium sulfate precipitated histones on Sephadex G-100 SF.

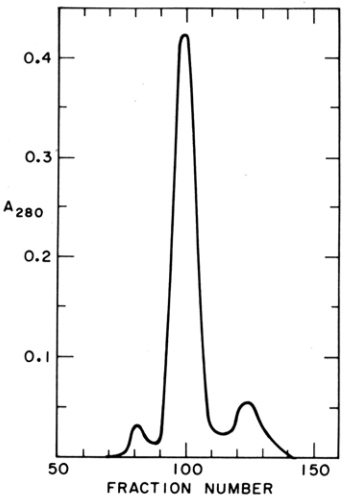

FIGURE 2: Gel filtration profile of ammonium sulfate precipitated histones on Sephadex G-100 SF. Approximately 4 mL of proteins was loaded on a 2.5 × 90 cm column equilibrated with 50 mM sodium bisulfite–50 mM sodium acetate–10 mM Na_2EDTA, pH 5.2. Flow rate = 5 mL/h; fraction volume = 2.5 mL/tube. The largest peak in the profile contains the arginine-rich histone proteins.

find that the tetramer binds to DNA to form a 1:1 (molar) protein/DNA complex which is highly elongated with approximate dimensions 450 × 80 × 80 Å and 1.5 superhelical turns. Addition of the lysine-rich histones to this particle forms a much more compact particle with physical properties nearly identical with those of a native nucleosomal core particle.

Materials and Methods

Preparation of Chromatin. Freshly excised calf thymus glands were immediately frozen in liquid nitrogen, transported under dry ice, and stored at −40 °C until use. Chromatin was isolated by a modification of the procedure of Zubay & Doty (1959). Thymus (30–40 g) and 0.5 mL of capryl alcohol were blended for 2 min at 80 V and 3 min at 45 V in a 790-W Waring blender containing 50 mM sodium bisulfite–75 mM sodium chloride–24 mM Na_2EDTA, pH 8. After filtration through cheesecloth, the solution was centrifuged at 400*g* for 10 min, and the resulting precipitate was reblended with 0.25 mL of capryl alcohol (5 s at 80 V, 25 s at 45 V) and centrifuged for four cycles in the same buffer and two cycles in a buffer of 0.3 M sodium chloride–10 mM sodium bisulfite–10 mM sodium acetate–10 mM Na_2EDTA, pH 5.2. The white chromatin pellet was then dissolved in 100 mL of 50 mM sodium bisulfite–50 mM sodium acetate, pH 5.2.

Preparation of Arginine-Rich Histones. The arginine-rich histones were isolated from chromatin by a modification of the procedure of Van Der Westhuyzen & Von Holt (1971). One hundred milliliters of 4.0 M sodium chloride–50 mM sodium bisulfite–50 mM sodium acetate, pH 5.2, was gradually added to the chromatin solution with gentle stirring. The viscous chromatin gel was combined with an equal volume (200 mL) of protamine sulfate (20 mg/mL) in the same buffer and the DNA was precipitated as a nucleoprotamine complex upon reduction of the sodium chloride concentration to 0.15 M during a 3-h dialysis. The proteins were concentrated by ultrafiltration (Amicon) and histones were separated from excess protamine by gel filtration on Sephadex G50 Superfine. Ammonium sulfate precipitation of the histones at 70% saturation removes histone H1 and much of the slightly lysine-rich histones, resulting in a precipitate selectively enriched in the arginine-rich histones (Figure 1). Further fractionation was then achieved by gel filtration on Sephadex G-100 Superfine (Figure 2) and the proteins were concentrated by a second ammonium sulfate precipitation. The histones thus obtained are electrophoretically pure (Figure 1); a molecular weight of 54 000 was obtained from sedimentation equilibrium ($\bar{v}$ = 0.74). Assuming an extinction coefficient at 275 nm for the tetramer of 1.88×10^4 M^{-1} cm^{-1} (D'Anna & Isenberg, 1974b), 20–25 mg of tetramer was obtained from approximately 40 g of calf thymus. All preparative procedures were performed at 4 °C unless stated otherwise.

Preparation of DNA. DNA fragments (140 base pair) were obtained from nucleosome core particles derived from H1-depleted chromatin (Klevan & Crothers, 1977). DNA was extracted from these particles by incubation at 37 °C with 100 μg/mL proteinase K (E. Merck) in 1 M NaCl for several hours followed by dilution to 0.6 M NaCl and precipitation with 2 volumes of 95% ethanol. The precipitate was redissolved in 50 mM Tris–10 mM Na_2EDTA, pH 7.6, extracted with buffered phenol and concentrated by a second ethanol precipitation. DNA was extracted from the redigested $(H3/H4)_2$–DNA particle by an identical procedure, except for omission of the phenol extraction.

Reconstitution Procedure. The arginine-rich histone tetramer was reconstituted to approximately 200 μg of 140 base pair DNA by a discontinuous salt gradient dialysis starting with 2.0 M NaCl–10 mM Tris–5 mM Na_2EDTA, pH 7.6 (2–4 h), followed by decreasing NaCl concentrations of 1.0 M (12 h), 0.8 M (10–12 h), 0.6 M (7–10 h), and 0.0 M (15–24 h). The final reconstituted particles in 2.0 mL of 10 mM Tris–5 mM Na_2EDTA, pH 7.6, were diluted fourfold for the electric dichroism experiments. Lysine-rich histones were reconstituted to the arginine-rich tetramer–DNA complex in 10 mM Tris–5 mM Na_2EDTA by dropwise addition of a concentrated protein solution (Camerini-Otero & Felsenfeld, 1977b). A molar extinction coefficient of 2.15×10^4 M^{-1} cm^{-1} was assumed for a tetramer $(H2A/H2B)_2$ of the lysine-rich histones (D'Anna & Isenberg, 1974b).

Analytical Procedures. Polyacrylamide gel electrophoresis of reconstituted particles and protein-free DNA was performed as described by Maniatis et al. (1975) using either 3.5 or 5% slab gels in 1/10 or 1/4 Tris–boric acid–EDTA (TBE) buffer. Gels 17 × 17 × 0.3 cm were run at 120 V (room temperature), stained with 5 μg/mL ethidium bromide, and photographed under UV light. Histone proteins were analyzed by electrophoresis in 15% acrylamide–2.5 M urea disc gels at pH 2.5 as described by Panyim & Chalkley (1969). Samples were dis-

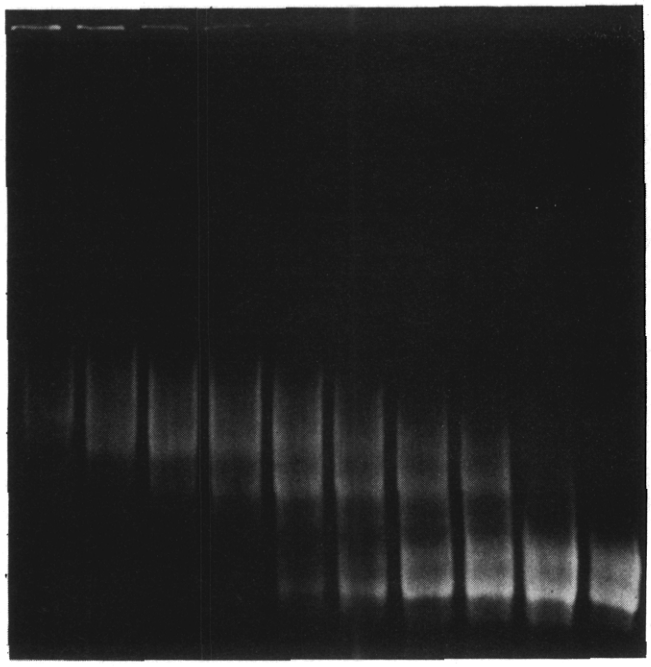

FIGURE 3: Polyacrylamide gel, 3.5%, in 1/10 TBE buffer or purified nucleosome DNA fragments reconstituted with the arginine-rich histones at several protein/DNA ratios. From right to left the molar ratio of $(H3/H4)_2$ to DNA fragments is: 0, 0.2, 0.4, 0.6, 0.85, 1.0, 1.25, 1.5, 2.0, and 2.5. The electrophoretic mobility of the equimolar protein/DNA particle is nearly identical with that of a nucleosome core particle (not shown).

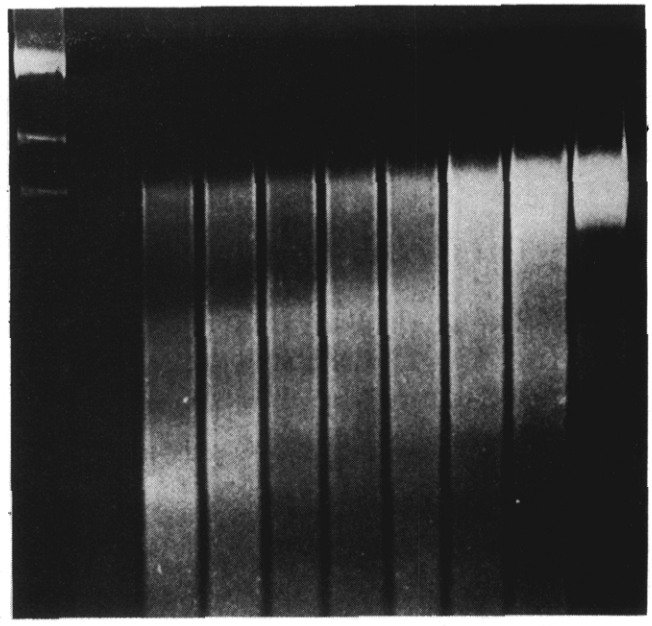

FIGURE 4: Five percent polyacrylamide gel in 1/4 TBE buffer of DNA from the equimolar $(H3/H4)_2$–DNA complex redigested with micrococcal nuclease. Reconstituted particles, 970 μL (approximately 1 A_{260}), in 10 mM Tris–0.025 mM Na_2EDTA, pH 7.6, was combined with 10 μL of 0.05 M $CaCl_2$ and 20 μL of nuclease (3000 U/mL) at room temperature. Digestion times from right to left are: 0, 0.5, 1, 2, 2.5, 3, 4, and 5 min. The final column is an *Hae* III digest of colicin E1 DNA used as a calibration standard.

solved in 8 M urea–0.5 M β-mercaptoethanol and allowed to stand for several hours prior to electrophoresis.

Ultracentrifugation studies were performed on a Spinco Model E analytical ultracentrifuge equipped with a photoelectric scanning system and an RTIC temperature control. Scans were taken at 266 nm and 4 °C of solutions containing 0.5–0.75 A_{260}. Circular dichroism measurements were obtained at room temperature in a Cary 61 recording spectropolarimeter taking $\epsilon_{260} = 6600\ M^{-1}\ cm^{-1}$ in terms of phosphate for calf thymus DNA.

Electric dichroism measurements were performed on a modified temperature jump apparatus as described elsewhere (Hogan et al., 1978). Samples (1.5 mL) containing approximately 0.5 A_{260} were subjected to field strengths not exceeding 40 kV cm^{-1}. Several successive traces were averaged for each measurement of the reduced dichroism or rotational correlation time. The rotational correlation time was determined by the viscosity enhancement technique in which a 10% (w/v) stock solution of Dextran T500 (Pharmacia) is diluted with sample to the desired concentration.

Results

Reconstitution Studies. The arginine-rich histone tetramer was reassociated with 140 base pair DNA fragments and analyzed by electrophoresis on polyacrylamide gels. A displacement of fluorescent intensity from the region of unbound DNA to a region of lower electrophoretic mobility is associated with an increase in the protein to DNA ratio (Figure 3). At approximately equal molar concentrations of protein and DNA, bands corresponding to free DNA are no longer present in the polyacrylamide gel. The reconstitution mixture for the particular experiment shown in Figure 3 contains a significant proportion (~50%) of 180 base pair DNA fragments which migrate above the nucleosome core DNA. Preferential binding of the histone tetramer to these larger DNA fragments is demonstrated by the relatively rapid disappearance of this band in comparison to the 140 base pair DNA band. Addition of histones in excess of a 1:1 molar ratio results in a further reduction in electrophoretic mobility and a disappearance of the lower of the two particle bands. There is also an increase in aggregated material which fails to enter the gel. The appearance of a sharp band corresponding to each species of DNA present in the reconstitution mixture and the disappearance of the free DNA bands at an equimolar protein/DNA ratio suggest the formation of a unique tetramer–DNA complex at the protein/DNA ratio found for these histones in the nucleosome.

The protein/DNA ratio in the reconstituted particles was determined by the method of Lowry et al. (1951). Adopting the nucleosome core particle as a calibration standard and assuming a protein/DNA weight ratio of 1.17, molar ratios of 0.9 and 0.4 were obtained for molar input protein/DNA ratios of 1.0 and 0.5, respectively. When redigested with micrococcal nuclease, the equimolar tetramer–DNA complex was degraded to broad DNA bands centered at approximately 85, 75, 55, and 45 base pairs (Figure 4). These DNA bands resemble the lower half of the DNA fragments produced in the limit digest pattern of chromatin and suggest an organization of DNA within the particle that is similar to that in a nucleosome core particle. These results are in qualitative agreement with those of Camerini-Otero et al. (1976).

Ultracentrifugation and CD Studies. Sedimentation equilibrium experiments were performed on the reconstituted tetramer–DNA complex at several input protein/DNA ratios. The apparent molecular weight, $MW_{(app)}$, is defined as:

$$MW_{(app)} = \frac{2RT}{\omega^2} \frac{d \ln A}{dr^2} \quad (1)$$

in which A is the absorbance of the solution, ω is the angular velocity, and r is the distance from the center of rotation. For a multicomponent system in which all species have identical

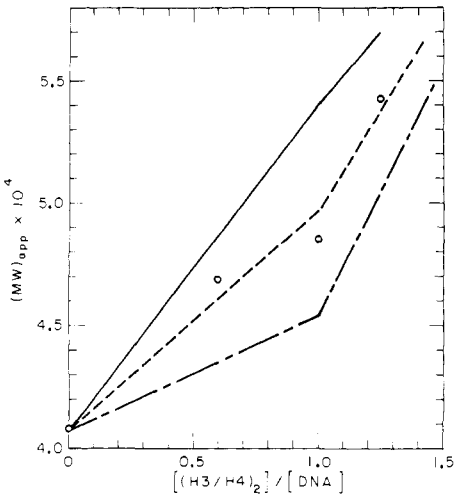

FIGURE 5: Sedimentation equilibrium apparent molecular weights of the reconstituted $(H3/H4)_2$-DNA particles at several protein/DNA molar ratios. Theoretical curves are presented assuming a partial specific volume, $\bar{v}$, of 0.62 (—), 0.65 (- - -), and 0.68 (- · - ·) for the equimolar tetramer-DNA complex. The apparent molecular weight $MW_{(app)}$ is defined as $MW(1 - \bar{v}\rho)$, where ρ is the solution density. Conditions: 10 mM Tris, 5 mM EDTA, pH 7.6, 4 °C.

extinction coefficients, the apparent molecular weight may be represented by:

$$MW_{(app)} = \frac{\Sigma(MW)_i C_i(1 - \bar{v}_i\rho)}{\Sigma C_i} \quad (2)$$

in which C_i and $\bar{v}_i$ are the molar concentration and partial specific volume of species i, respectively. Assuming a $\bar{v}$ of 0.54 for DNA, a molecular weight of 8.84×10^4 was determined by sedimentation equilibrium, corresponding to approximately 132 base pairs.

We have fitted our ultracentrifugation results to a stepwise association model in which all protein is bound as a unique $(H3/H4)_2$-DNA particle when reassociated in a molar tetramer concentration less than or equal to that of DNA. When DNA is reconstituted with an excess of histones, an additional particle can form with a protein/DNA ratio identical with that of a nucleosome. This model is strongly suggested by the gel electrophoresis results in Figure 3. A value for $\bar{v}$ of 0.661 was assumed for the particle containing two histone tetramers (Olins et al., 1976). Agreement between the experimental and theoretical results presented in Figure 5 suggests a $\bar{v}$ of 0.65 for the equimolar tetramer-DNA complex, significantly larger than the value calculated by addition of molar volumes of the two components. Thus the equilibrium sedimentation results are consistent with the stepwise association model, although taken by themselves they do not prove the model.

The sedimentation coefficient $s_{20,w}$ of the reconstituted $(H3/H4)_2$-DNA particle at several protein/DNA ratios is presented in Figure 6. A $s_{20,w}$ of 6.45 was observed for the 1:1 molar complex, with a linear rise in sedimentation coefficient at higher protein/DNA ratios. At a molar ratio of 2.4 the sedimentation coefficient is 8.75, again suggesting that additional protein can bind to the equimolar tetramer-DNA complex. A sedimentation coefficient of 6.45 and a partial specific volume of 0.65 define a ratio of translational functional coefficients, f/f_0, of 2.1 for the equimolar complex. This implies a highly asymmetric and/or highly hydrated particle.

The circular dichrosim spectrum of the $(H3/H4)_2$ tetramer reconstituted to DNA at several protein/DNA ratios was measured in the wavelength range of 260 to 320 nm, in which histones have little absorbance. The measured molar ellip-

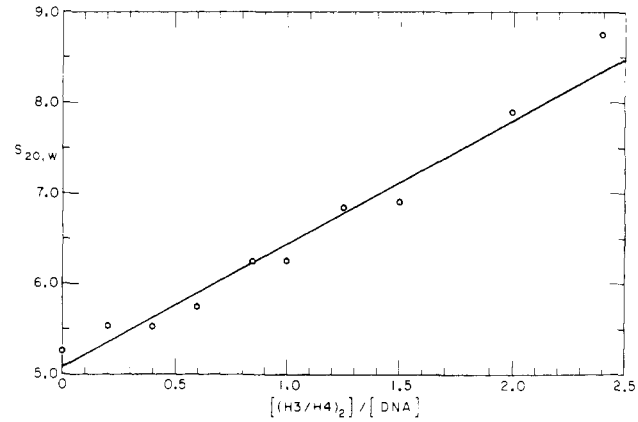

FIGURE 6: Sedimentation coefficients, $s_{20,w}$, of 140 base pair DNA fragments reconstituted to the arginine-rich histones at several protein/DNA molar ratios. Scans were recorded at 25 980 rpm and 4 °C.

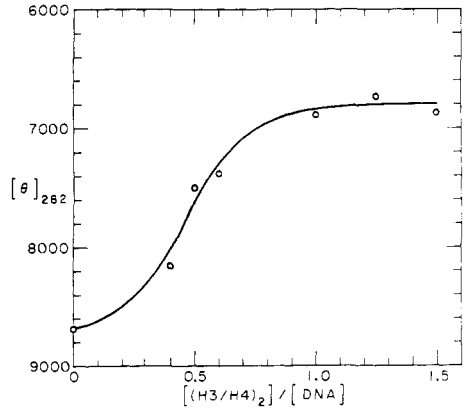

FIGURE 7: The molar ellipticity at 282 nm, $[\Theta]_{282}$, of 140 base pair DNA fragments reconstituted to the arginine-rich histone tetramer at several molar protein/DNA ratios, T = 20 °C.

ticities at 282 nm for protein-free DNA and the 1:1 protein/DNA complex were 8680 deg cm^2/dmol of phosphate and 6888 deg cm^2/dmol of phosphate, respectively (Figure 7). For comparison, the molar ellipticity of nucleosome core particles obtained under identical conditions is 1800 deg cm^2/dmol of phosphate (Klevan & Crothers, 1977). The leveling off of the ellipticity between a 1:1 and 1.25:1 protein/DNA ratio is further evidence for formation of a unique $(H3/H4)_2$-DNA complex.

Electric Dichroism Studies. The conformation of DNA in the reconstituted $(H3/H4)_2$-DNA complex was investigated by the technique of electric dichroism. DNA is both optically and electronically anisotropic, exhibiting a maximum extinction coefficient for light polarized perpendicular to its helical axis when oriented in an electric field (Ding et al., 1972). The reduced dichroism, ρ, is defined as (Fredericq and Houssier, 1973)

$$\rho = \frac{A_\parallel - A_\perp}{A} = \frac{1.5(A_\parallel - A)}{A} \quad (3)$$

in which $A_\parallel$ and $A_\perp$ refer to light polarized parallel and perpendicular to the electric field and A is the absorbance in the absence of orientation. For DNA, the reduced dichroism may be related to the angle α between the orienting electric field and the electronic transition moment, which lies in the plane of the bases:

$$\rho = 1.5(3\cos^2\alpha - 1)\Phi \quad (4)$$

Φ being a field dependent orientation function, $0 \leq \Phi \leq 1$

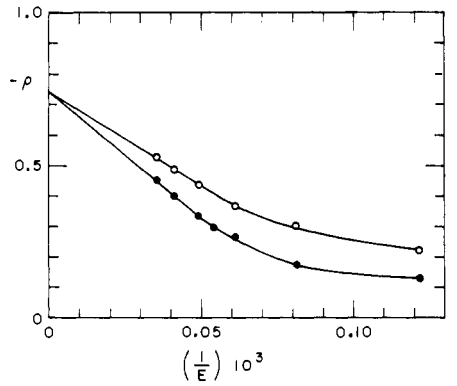

FIGURE 8: The reduced dichroism ($-\rho$) as a function of inverse electric field strength ($1/E$) for the equimolar tetramer-DNA particle in 2.5 mM Tris-1.25 mM Na_2EDTA (●) and 1 mM Tris-0.5 mM Na_2EDTA (○), pH 7.6, T 7 °C.

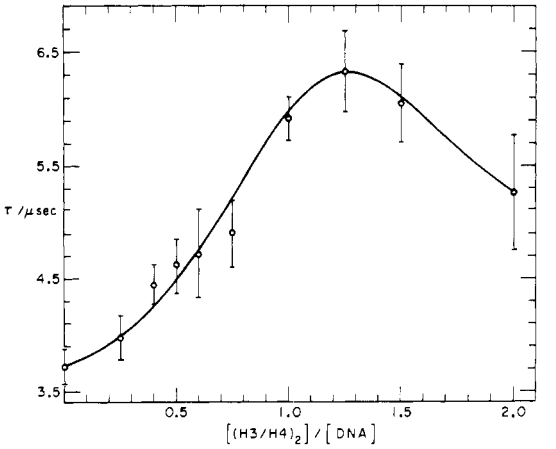

FIGURE 9: Rotational correlation time τ as a function of $[(H3/H4)_2]/[DNA]$ ratio. Error bars represent 95% confidence limits derived from the reconstitution of three separate protein and DNA preparations. Each point is representative of several electric field measurements from 10 to 30 kV; T = 7 °C, 2.5 mM Tris-1.25 mM Na_2EDTA, pH 7.6.

(O'Konski et al., 1959). If DNA is in the form of a superhelix, then the reduced dichroism is a function of the pitch angle, β, which is defined by the equation

$$\tan \beta = \frac{2\pi r}{P} \tag{5}$$

where r and P are the radius and pitch of the superhelix, respectively. The equation for the reduced dichroism of a superhelix is then (Rill, 1972):

$$\rho = 0.75(3 \cos^2 \alpha - 1)(3 \cos^2 \beta - 1) \tag{6}$$

An angle α of 73 ± 2° has been measured for a number of DNA fragments in the size range of 100–250 base pairs under a variety of ionic conditions (Hogan et al., 1978). Assuming a similar value of α in the protein-DNA complex, an extrapolation of the reduced dichroism to infinite electric field (Φ = 1) yields a value for the pitch angle of the superhelix.

The field extrapolation of the reduced dichroism for the equimolar $(H3/H4)_2$-DNA complex is shown in Figure 8. The linear dependence of ρ on E^{-1} at high electric fields and the dependence of the field extrapolation on ionic strength (a steeper slope corresponding to a lower apparent dipole moment) is similar to the behavior observed for DNA under identical conditions (Hogan et al., 1978), and in contrast with the properties of nucleosomes (Crothers et al., 1978), which appear to orient because of a permanent molecular dipole moment. Therefore, it appears that like DNA the tetramer-DNA complex orients because the electric field induces an apparent dipole moment. However, the reduced dichroism at infinite orientation is found from Figure 7 to be −0.74, significantly less than the value of −1.1 to −1.2 observed for 140 base pair DNA fragments.

The time course for orientation in an electric field of a molecule lacking a permanent dipole moment is given by (Tinoco, 1955);

$$\rho_{(t)} = \rho_{(\infty)}(1 - e^{-t/\tau}) \tag{7}$$

in which $\tau = 1/6D_r$ and D_r is the rotational correlation time for reorientation along the principal axis of the molecule. DNA fragments (140 base pair) have a rotational correlation time of approximately 3.7 μs, whereas nucleosomes exhibit a τ of 0.8 ± 0.2 μs (Klevan et al., 1977). When the arginine-rich histones are reconstituted to 140 base pair DNA fragments, τ increases to a maximum value of 6.3 μs at a protein/DNA ratio of 1.25:1 (Figure 9). This result demonstrates that the H3-H4 tetramer-DNA complex is much less compact than a nucleosome. All orientation curves could be fitted to a single exponential function, eq 7.

As the protein/DNA ratio increases further, τ decreases (Figure 9). Generally it was necessary to clarify the solutions in which the molar concentration of tetramer was greater than that of DNA. The particle reconstituted at an input 1:1 molar $(H3/H4)_2$/DNA ratio was centrifuged through a 5–20% sucrose gradient containing 0.1 M NaCl-10 mM Tris-1 mM Na_2EDTA, pH 7.6, in an SW40 rotor (35 000 rpm) for 21 h at 4 °C. A single symmetric peak was obtained with a rotational correlation time and reduced dichroism identical with the starting material. In agreement with the results presented earlier, a unique complex appears to be formed at a 1:1 molar tetramer/DNA ratio.

The electric dichroism results place considerable constraint on possible structural models for the tetramer-DNA particle since the model must fit both the dichroism amplitude and the rotational correlation time. The equation of Broersma (1960) for the rotational diffusion constant of a rod has been used to model the protein-DNA complex:

$$D_r = \frac{3kT}{\pi\eta L^3}\left\{\ln\left(\frac{L}{b}\right) - 1.57 + 7\left(\frac{1}{\ln(L/b)} - 0.28\right)^2\right\} \tag{8}$$

in which L is the length of a rod of radius b, η is the solvent viscosity, and kT is the Boltzmann constant times temperature. Assuming 5 Å of rotationally bound water and α equal to the value measured for DNA, a molecule containing 1.5 superhelical turns and dimensions of 450 × 83 × 83 Å would have a rotational correlation time of 6.3 μs and reduced dichroism of −0.74, in agreement with the experimental values.

If the molecule possessed a permanent dipole moment, then the longest correlation time would be given by $\tau = \frac{1}{2}D_r$, requiring a smaller particle. The only model of appropriate size we have found whose dichroism approaches the experimentally observed value is a disk about 200 Å across, with ¾ turn of DNA wound around it. When oriented with its C_2 axis parallel to the field this structure should have a dichroism of about −0.6 (Crothers et al., 1978). Because of the dependence of the apparent dipole moment on salt concentration, we prefer the first model in which the apparent dipole moment is induced by the field, producing orientation of the 450-Å long superhelix with its C_2 axis perpendicular to the field.

Stepwise Reassociation of Nucleosome Core Particles.

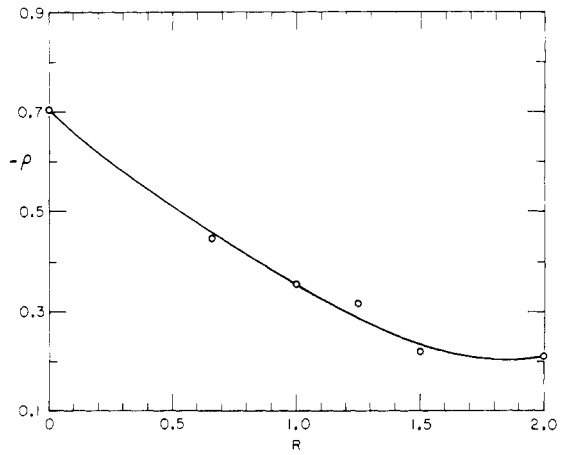

FIGURE 10: Reduced dichroism as a function of added lysine-rich histone concentration for the equimolar $(H3/H4)_2$-DNA particle. R is defined as $[(H2A/H2B)_2]/[(H3/H4)_2\text{-DNA}]$; T = 7 °C, 2.5 mM Tris-1.25 mM Na_2EDTA, pH 7.6.

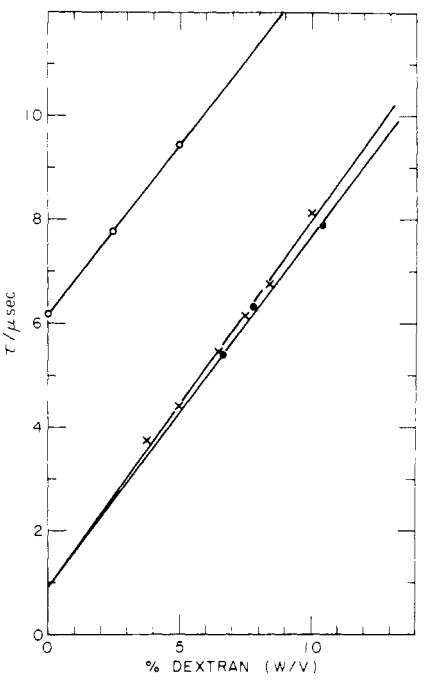

FIGURE 11: Rotational correlation time τ as a function of added dextrans concentration for the equimolar $(H3/H4)_2$-DNA particle (○), nucleosome core particle (X), and the particle formed by direct addition of lysine-rich histones to the preformed arginine-rich histone 140 base pair DNA complex (●); T 7 °C, 2.5 mM Tris-1.25 mM Na_2EDTA, pH 7.6.

Addition of the lysine-rich histones to a solution of $[(H3/H4)_2]_1[DNA]_1$ particles results in the formation of a new particle with hydrodynamic properties nearly identical with that of a nucleosome. This occurs without gradient dialysis or prior removal of the more tightly bound arginine-rich histones. The variation in the reduced dichroism of the equimolar $(H3/H4)_2$-DNA particle when reconstituted with lysine-rich histones is shown in Figure 10. Here R represents the molar ratio of a tetramer equivalent of the lysine-rich histones $(H2A/H2B)_2$ reconstituted to the $(H3/H4)_2$-DNA particle; R is equal to unity for the protein/DNA ratio of a nucleosome. It is evident that $-\rho$ decreases from an initial value of 0.74 at zero R to a value of approximately 0.22 at an R of 1.5. The value of $-\rho$ observed for the nucleosome core particle extrapolated to infinite field under identical conditions was 0.28. This decrease in $-\rho$ with increasing R corresponds to an increase in the superhelical pitch angle and the formation of a more condensed structure.

It has been demonstrated (Hogan et al., 1978) that the rotational correlation time τ varies in a linear fashion with the concentration of an added neutral polymer (dextran). Therefore, it is possible to measure the rise kinetics in an electric field of particles which orient with a time constant faster than the 1-2 μs limitation of the instrumentation. This viscosity enhancement technique applied to nucleosomes and the $[(H3/H4)_2]_1[DNA]_1$ particle is shown in Figure 11. The value of τ extrapolated to zero dextran is 0.8 μs for the nucleosome core particle and 6.15 μs for the reconstituted arginine-rich tetramer-DNA complex. The latter number is equal to the value actually measured in dextran-free solution. When the lysine-rich histones are reconstituted to this histone-DNA complex in a $[(H3/H4)_2]_1[(H2A/H2B)_2]_{1.5}[DNA]_1$ ratio, the particle which forms is nearly indistinguishable from a native nucleosome particle in its rotational correlation time. If the input lysine-rich histone concentration exceeds a R of 2.0, a precipitate forms in the reconstitution mixture.

The sedimentation coefficient $s_{20,w}$ of the reconstituted nucleosome particle is 9.6 S in a buffer of 10 mM Tris-5 mM Na_2EDTA, pH 7.6. Native nucleosome core particles exhibit a $s_{20,w}$ of 10.3 under identical conditions.

Discussion

We have reported the isolation and characterization of a stable particle formed from the arginine-rich histone tetramer and 140 base pair DNA. In agreement with Bina-Stein & Simpson (1977), our results are consistent with the preferential binding of one tetramer to each 140 base pair DNA fragment to form an equimolar protein-DNA complex. This conclusion is supported by the observation that (a) bands corresponding to free DNA are no longer present when particles reconstituted in a 1:1 molar ratio are examined on polyacrylamide gels, (b) the decline in molar ellipticity at 282 nm, $[\Theta]_{282}$, plateaus at a 1:1 molar complex, and (c) the rotational correlation time τ reaches a maximum of 6.3 μs at a protein/DNA ratio of 1.25:1.

The rotational correlation time is proportional to the rotational frictional coefficient and is therefore expected to decrease with the induction of supercoiling in DNA. There is, however, a large increase in the hydrodynamic volume when the $(H3/H4)_2$ tetramer binds to DNA and this is reflected by an increase in τ with increasing protein concentration (Figure 9). A rotational relaxation time of 6.3 μs and a reduced dichroism of −0.74 are consistent with a highly elongated structure for the tetramer-DNA complex with overall dimensions of approximately 450 × 80 × 80 Å and 1.5 superhelical DNA turns.

Recently, Camerini-Otero et al. (1977) reported that reconstitution of the $(H3/H4)_2$ tetramer to DNA in a 0.75 g/g of protein/DNA ratio gives rise to two discrete particles with sedimentation coefficients of 7.25 and 9.75. The particles had protein/DNA ratios of 0.6 g/g and 0.91 g/g, suggesting the presence of one and two bound tetramers for the slower and faster moving particles, respectively. Our observations are in agreement with their results. It appears that at high protein/DNA ratios a more compact particle than the equimolar $(H3/H4)_2$-DNA complex can form, giving rise to a reduction in the rotational correlation time and an increase in the sedimentation coefficient. We have not isolated this particle in our study, but the data suggest it might be the 9.7S particle of Camerini-Otero et al. (1976).

Addition of the lysine-rich histones to the equimolar

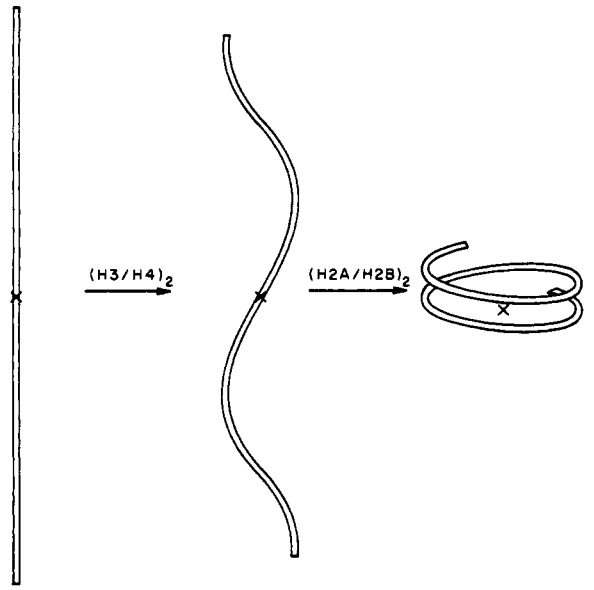

FIGURE 12: A model for the induction of supercoiling in 140 base pair DNA fragments by the arginine-rich and lysine-rich histones. The dyad axis in the molecule is indicated by "X".

(H3/H4)-DNA complex results in the formation of a particle hydrodynamically equivalent to a nucleosomal core particle. Camerini-Otero & Felsenfeld (1977b) have demonstrated that DNA "primed" with the arginine-rich histones and reconstituted by direct addition with the lysine-rich histones will reproduce the micrococcal nuclease limit digest pattern of chromatin. When we reconstitute the arginine-rich histones into 140 base pair DNA in an analogous manner, the rotational correlation time, reduced dichroism, and sedimentation coefficient of the resulting particle suggest a folding of the DNA equivalent to that in a nucleosome. Furthermore, the extended nature of the $(H3/H4)_2$-DNA complex is not the result of ineffective reconstitution, since addition of the lysine-rich histones reforms a nucleosome-like particle. Histones were reconstituted most effectively at a relative molar ratio $[(H3/H4)_2]_{1.25}[(H2A/H2B)]_{1.5}[DNA]$. The slight excess of histones needed to reconstitute the nucleosome particle might be due to loss of protein during the reconstitution procedure, as suggested by the Lowry protein determinations and as reported by Bina-Stein & Simpson (1977).

There is evidence that the nucleosome particle contains a dyad axis (Finch et al., 1977) and that a dimer of the H3 proteins lies near this axis (Camerini-Otero & Felsenfeld, 1977a). The salt concentration dependence of the apparent dipole moment of the$(H3/H4)_2$-DNA complex indicates the absence of an appreciable permanent dipole moment in this particle. Furthermore, when redigested with micrococcal nuclease the 140 base pair DNA is rapidly digested to DNA bands in the range of 70–80 base pairs, suggesting a possible central cleavage site in the molecule. Therefore, it appears that the $(H3/H4)_2$-DNA complex might also contain a dyad symmetry axis with symmetric disposition of histones along the superhelical axis. Alignment in an electric field with the superhelical axis parallel and the dyad axis perpendicular to the field would account for the lack of apparent permanent dipole moment.

A model for the condensation of DNA induced by the stepwise reassociation of the histone proteins is presented in Figure 12. Reconstitution of the arginine-rich histone tetramer to 140 base pair DNA results in a gentle supercoiling of the DNA with a superhelical pitch angle of approximately 28°. A tetramer (or two dimers) of the lysine-rich histones completes the structure to form a nucleosome core particle. The dyad axis of the DNA, indicated by a symbol "X" in Figure 12, is conserved during the reconstitution procedure. The absence of a permanent moment in the molecule coupled with the presence of H3 near the dyad axis in nucleosomes suggests that the H3 protein dimer is situated in this region in the $(H3/H4)_2$-DNA particle.

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Protein Analysis of Cardiac Sarcolemma: Effects of Membrane-Perturbing Agents on Membrane Proteins and Calcium Transport†

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ABSTRACT: Protein composition of cardiac sarcolemmal membranes was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Membranes were observed to contain about 20 polypeptide bands ranging from 18 000 to 200 000 dalton mass. Out of these, six bands were prominent and together comprised 57% of the membrane protein. When sarcolemmal membranes, phosphorylated by [γ-^{32}P]ATP in the presence of Ca^{2+} or Na^+ with and without K^+, were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis at pH 2.4, the band III region (M_r 105 000) of gels was found to contain active sites of monomeric Ca-ATPase and (Na,K)ATPase. Bands I (M_r > 200 000), II (M_r 150 000), III (M_r 105 000), and VI (M_r 47 000) were accessible to trypsin; the extent of proteolysis was dependent on the time of exposure to, and the concentration of, trypsin (i.e., ratio of sarcolemmal protein/trypsin). Addition of molar sucrose protected sarcolemmal proteins from the tryptic proteolysis. Calcium transport was reduced by the action of trypsin; the degree of reduction was influenced by the time of exposure of membranes to trypsin as well as the concentration of trypsin. (Mg,Ca)ATPase activity, on the other hand, was elevated moderately at lower concentration and reduced at higher concentration of trypsin. Treatment with phospholipase C caused a time- and concentration-dependent decrease in calcium transport and (Mg,Ca)ATPase activity; electrophoretic patterns were unaffected by this treatment. Addition of lecithin to phospholipase C treated membranes produced a moderate increase in calcium transport. Exposure to Triton X-100 (1%) specifically solubilized three protein bands (M_r 90 000, 67 000, and 57 000), whereas exposure to deoxycholate (1%) preferentially solubilized high-molecular-weight proteins, including band III (M_r 105 000); Lubrol-PX (1%) caused nonspecific solubilization of proteins, although the extent of solubilization with Lubrol-PX was considerably less than with either Triton or deoxycholate.

It is recognized that cardiac contractility is regulated by several hormones and affected by drugs, which are thought to interact with specific receptors located on the sarcolemmal membranes (plasma membranes), as well as by transsarcolemmal ion fluxes. In our laboratory, we have initiated a series of biochemical investigations of cardiac sarcolemma with the ultimate aim of understanding initial molecular events occurring in these membranes due to cholinergic-adrenergic hormone interactions with the cardiac muscle. We have reported a procedure for the isolation of cardiac sarcolemma in a high degree of purity (Sulakhe et al., 1976a) and have characterized these membranes both biochemically and morphologically (St. Louis and Sulakhe, 1976a). Heart sarcolemmal membranes were observed to contain a calcium-transport system (Sulakhe et al., 1976a), whose properties were studied in some detail (St. Louis and Sulakhe, 1976b) and which was regulated by cAMP[1]-dependent phosphorylation of sarcolemmal proteins (Sulakhe et al., 1976a; Sulakhe and St. Louis, 1978; St. Louis and Sulakhe, in preparation). Considerable amounts of adenylate and guanylate cyclase were present in these membranes (Sulakhe et al., 1976b), and the activities of these enzymes were increased moderately by β-adrenergic and cholinergic agents, respectively (St. Louis and Sulakhe, 1976c; Narayanan and Sulakhe, manuscript in preparation); specific β-adrenergic receptors (Narayanan and Sulakhe, unpublished work) and cholinergic receptor sites were also present (Ma et al., 1978; Wei and Sulakhe, in preparation).

Analysis of proteins of sarcolemma and their biochemical functions is of potential significance in understanding molecular aspects of ion-transport systems and of receptor(s)-cyclase

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[1] Abbreviations used: cAMP, cyclic adenosine 3′,5′-monophosphate; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; POPOP, 1,4-bis[2-(5-phenyloxazolyl)]benzene; PPO, 2,5-diphenyloxazole; Cl_3AcOH, trichloroacetic acid; $NaDodSO_4$, sodium dodecyl sulfate; Tris-Cl, 2-amino-2-hydroxymethyl-1,3-propanediol chloride.

0006-2960/78/0417-4540$01.00/0