Participation of Core Histone "Tails" in the Stabilization of the Chromatin Solenoid

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ABSTRACT We show here that the solenoid is maintained by the combination of linker histones and the nonglobular, highly basic "tails" of the core histones, which play only a minor part in the formation of the nucleosome core (Whitlock and Simpson, 1977. *J. Biol. Chem.* 252:6,516–6,520; Lilley and Tatchell, 1977. *Nucleic Acids Res.* 4:2,039–2,055; and Whitlock and Stein, 1978. *J. Biol. Chem.* 253:3,857–3,861). Polynucleosomes that contain core histones devoid of tails remain substantially unfolded under conditions otherwise favorable for the formation of solenoids. The tails can be replaced by extraneous basic polypeptides and in the presence of the linker histones the solenoid structure is then spontaneously recovered, as judged by a wide variety of structural criteria. The inference is that the core histone tail segments function by providing electrostatic shielding of the DNA charge and at the same time bridging adjacent nucleosomes in the solenoid. Our results carry the further implication that posttranscriptional modifications, such as acetylation of ϵ-amino groups, that reduce the positive charge of the core histone tails will tend to destabilize the higher-order structure and could thus render the DNA with which they are associated more readily available for transcription.

The polynucleosome chain of chromatin can exist in at least two distinct states: in one the nucleosomes are disposed at regular intervals along an essentially flexible and disordered chain and in the other they form a higher order helix, referred to as the solenoid (4); this state has about six nucleosomes per turn and a pitch in the direction of the solenoid axis comparable with the principal dimension (11 nm) of the particles themselves (4–6). This results in a 50-fold contraction of the DNA relative to the unperturbed double helix. The solenoid must be disrupted during transcription and replication of the DNA. Furthermore, putative promoter sites for selective transcription lie within the size range (up to 240 base pairs) of the DNA segments that link successive nucleosomes; they could thus be buried in the higher order structure and thereby rendered inaccessible to regulatory proteins. An analysis of factors governing the equilibrium between the solenoid and the extended chain of chromatin is an important step towards an understanding of the mechanisms of transcriptional control.

The importance of the linker histone (H1 or one of its close analogues) for the formation of the solenoid has been recognized for some time (5, 7, 8). Chromatin denuded of H1 exists only in the extended state (5, 8); when the H1 is restored, the solenoid structure is regained to an extent proportional to the amount of H1 bound, reaching a limit (for chicken erythrocyte chromatin) at a stoichiometry of two molecules per nucleosome (8). Furthermore, one can recognize separate roles for the three structural domains of H1, the two unstructured terminal segments and the central globular domain (9), in the folding process. The globular region binds specifically to the nucleosome core and the tails promote the condensation of the DNA (9). The exchange of H1 between polynucleosome chains shows that this histone is dissociable (10), so that the DNA in the linker regions may be partially unmasked. How the sequence variants of H1 (e.g. the two species of H1 or H5 in chicken erythrocytes) or posttranslational modifications of the H1 (11) influence the stability of the higher order structure or the accessibility of the linker DNA is still not known.

The core histone octamer must also be supposed essential for stability of the higher order structure, at least insofar as the core histones are required for the attachment of H1. Whether the core histones play a further role in the induction of higher order structure of chromatin is the question examined here. The nature and specificity of interactions between the nucleosome cores in the solenoid have not previously been studied. Our object has been to investigate the function of the core histone "tails" in this structure. We have taken advantage of the ability of trypsin, acting on the depleted chromatin, to detach these tails uniquely (12–14). Recombination experiments were then performed on the resulting material.

We focus on the tails because their posttranslational modi-

fication, particularly acetylation (15–17), has been associated with transcriptional activity (18, 19). Removal of the entire tails may be seen as an extreme form of such modification, and it is relevant therefore to examine the effects of such treatment on chromatin structure.

MATERIALS AND METHODS

Chromatin

Native chromatin from chicken erythrocyte nuclei was prepared and fractionated into discrete size classes as previously described (8). H1- and H5-depleted chromatin (stripped chromatin or SPN) was prepared from native chromatin by passage over a DNA cellulose column (20). The average polynucleosome size of each fraction ($\bar{N}$) was determined from measurements of DNA lengths by agarose gel electrophoresis (one nucleosome was equal to 212 base pairs).

Proteins

The preparation of chicken erythrocyte H1 and H5, their reaction with *N*-succinimidyl propionate (The Radiochemical Centre, Amersham, England), and the methods used to determine their concentrations have been described previously (8). The COOH-terminal domain of calf thymus H1 (CH1) was prepared as previously described (9). Proteins were analyzed by electrophoresis in 18% SDS acrylamide gels (21).

Trypsin Digestion

Stripped polynucleosomes (SPN) were digested with trypsin (Type II, Sigma Chemical Co., Dorset, England), in 10 mM Tris/HCl, 0.1 mM EDTA, 80 mM NaCl, pH 7.5, at 4°C. Chromatin concentrations were between 1.0 and 2.0 A_{260} U/ml and digestion was done at a trypsin concentration of 0.5 μg/ml. The reaction, which was allowed to proceed for 4 h, was terminated by the addition of a fourfold excess of Soybean trypsin inhibitor (Type I-S, Sigma Chemical Co.). Trypsin-digested, stripped polynucleosomes (tryp.SPN) were dialyzed overnight against 10 mM Tris/HCl, 0.1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 80 mM NaCl, pH 7.5 (TEP_{80}) before further use.

Reconstitution

Reconstitution was carried out in TEP_{80} as previously described (8). For complexes containing CH1, an incubation of at least 1 h with this fragment alone was allowed before subsequent additions were made.

Nuclease Digestion

Micrococcal nuclease digestion and the analysis of the resulting DNA products in agarose or acrylamide gels followed earlier procedures (8).

Sedimentation

Sedimentation coefficients were determined in the Beckman Model E Ultracentrifuge using ultraviolet absorption optics (Beckman Instruments, Inc., Fullerton, CA). Analyses were carried out at a chromatin concentration of 0.7 A_{260} U/ml. in TEP_{80}. Some samples were analyzed by sedimentation in isokinetic sucrose gradients (5–27%) containing TEP_{80}. Centrifugation was carried out for 100–120 min at 40,000 rpm in a Beckman SW41 rotor at 4°C.

Electric Dichroism

The apparatus and techniques used have been described (6). Before measurement, reconstituted samples were dialyzed against 0.22 mM cacodylate, 5 μM EDTA, pH 7.0 (ED buffer), magnesium chloride being added subsequently if required. Measurements were made at a chromatin concentration of 0.25 A_{260} U/ml.

Electron Microscopy

The preparation of samples and the procedures for fixation, spreading, and shadowing have been described before (8).

RESULTS

The products of digestion of the core histones *in situ* in stripped chromatin (SPN) were analyzed by gel electrophoresis in the presence of SDS. The results are shown in Fig. 1. In agreement with previous studies (1–3, 22, 23), five prominent bands, designated P1–P5 (22), were found to be present. The parts of the histones eliminated by trypsin were degraded to small polypeptides, not detected in an 18% acrylamide gel. In TEP_{80} these fragments do not remain bound to the chromatin and are removed during the subsequent dialysis treatment (data not shown).

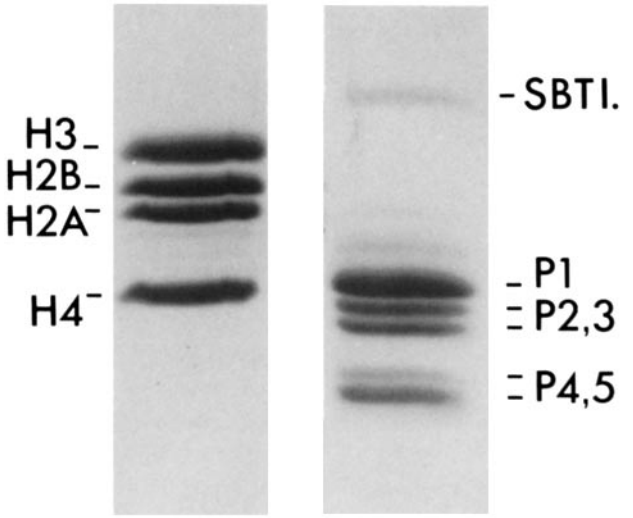

FIGURE 1 Histones from SPN (left) and tryp.SPN (right) analyzed in an 18% SDS acrylamide gel. *SBTI* is soyabean trypsin inhibitor. The nomenclature for the trypsin-resistant peptides is taken from reference 22.

Characterization of Trypsin-treated SPN

Micrococcal Nuclease Digestion

Tryp.SPN and SPN were digested with micrococcal nuclease and the DNA products were analyzed by electrophoresis in a 1% agarose gel (Fig. 2). Two points emerged. First, there was no change in the chromatin repeat (212 base pairs) and, second, no significant redistribution or sliding of the trypsin-resistant core histones had occurred during or after exposure to trypsin. Densitometer tracings from corresponding channels in Fig. 2 were completely superimposable. Analysis of products of more extensive digestion in 7% acrylamide gels (Fig. 3) revealed that the DNA of tryp.SPN was degraded to monomer and submonomer fragments about twice as rapidly as that in SPN. However, within the limits of resolution of these experiments, there was no significant difference in the nature of the submonomer bands generated from the two samples. Prominent bands were observed at 146, 135, 124, and around 103 base pairs.

Sedimentation

The isokinetic sucrose gradient sedimentation profiles shown in Fig. 4 reveal that tryp.SPN sediments more slowly than SPN. Table I gives the sedimentation coefficients (determined in the analytical ultracentrifuge) of tryp.SPN and the SPN from which they were derived. The loss of the core histone tails led to a diminution of ~20% in the sedimentation coefficient.

Electric Dichroism

The electric dichroism of chromatin (24–27) yields information relating to two structural features. First, the average

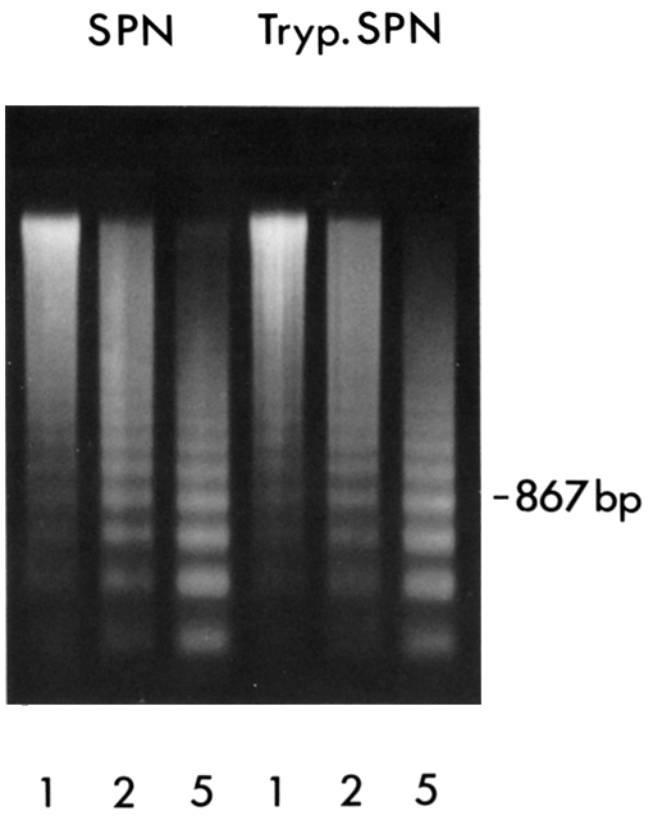

FIGURE 2 Agarose gel (1%) analysis of DNA isolated from micrococcal nuclease digests of *SPN* and *tryp.SPN*. Times of digestion (min) are shown below each track. The marker used for repeat length determination was an Hae III digest of PM2 DNA (not shown).

orientation of the DNA relative to the fiber axis can be estimated from the limiting reduced dichroism. Second, the relaxation rate of the fibers after the extinction of the orientating field can be measured and this depends on the rotational diffusion coefficient and hence on the dimensions and flexibility of the particle. These two parameters taken together form an exacting criterion for the structural state of chromatin (6, 27, 28).

Useful structural information can be obtained from electric dichroism only if the data can be extrapolated to infinite field strengths. Under the conditions used here and in the study by McGhee et al. (6), the dipole properties of both open and compacted chromatin closely resemble those observed for DNA (41). Classical equations for the dependence of the dichroism on the field strength, E, for a simple induced dipole moment do not adequately fit the data in the high field region. It is generally accepted that the induced dipole moments of polyelectrolytes like DNA saturate quickly with increasing E. Although there are a few semi-theoretical orientation functions available in the literature (41, 42, and Diekmann et al. manuscript submitted for publication) that attempt to describe the high field behavior of DNA, none is compelling. It is our belief that a simple quadratic fit of the high field ρ vs. $1/E$ data gives an extrapolated dichroism that is at least as meaningful as any theoretical fit and is more firmly rooted in the actual data.

As monitored by electric dichroism, there are three important criteria that characterize the transition of native chromatin from an open, extended form to a compact, folded structure. The extrapolated, infinite field dichroism at 260 nm, $\rho\infty$, decreases from about −0.54 to −0.21 (6), reflecting a change in the orientation of the DNA with respect to the long axis of the chromatin. Secondly, as chromatin folds up with added Mg^{++}, for example, there is a dramatic decrease in the relaxation time of the decay. Just as significantly, the character of the kinetics also changes substantially. The decay of dichroism for the open form is not described by a single exponential but rather consists of a large spectrum of relaxation times. This is the expected behavior if the open form is flexible and not rigidly held in a particular conformation. The average relaxation times we report here are calculated from the area under normalized decay curves and represent the average relaxation time as weighted by each component's contribution to the dichroism. For open chromatin, the longest relaxation time observed in a semilog plot is ~4–5 times larger than the area average. Once the chromatin folds up into a compact structure, however, 90–95% of the total decay can be well described by single exponential kinetics, which is consistent with the formation of a rigid structure with very little internal motion possible.

Fig. 5 shows the dependence of the reduced electric dichroism (ρ) on the reciprocal field strength for SPN in the absence (*a*) and for SPN (*b*) and tryp.SPN (*c*) in the presence of magnesium ions. Extrapolation to infinite field strength leads to the limiting values corresponding to full orientation. The results show that SPN has a limiting reduced dichroism ($\rho\infty$) of −0.77 in the absence and −0.49 in the presence of magnesium. With 70 μM Mg^{++} added ($[Mg^{++}]/[DNA\text{-}P] = 2$), the area tau was ~400 μs, at high fields, indicating that the sample was in an open, extended conformation even though native chromatin would have been compacted at this magnesium to DNA ratio, and the spectrum of relaxation times observed demonstrated that the chromatin remained very flexible.

Determining a limiting reduced dichroism for the tryp.SPN sample proved more difficult. In contrast to the SPN data, this sample never reached a well-defined linear region of ρ vs. $1/E$. Nonetheless, a quadratic fit to the data estimates that $\rho\infty \simeq -0.96$. In comparison with an observed $\rho\infty \simeq -0.54$ for native chromatin, it would be concluded that tryp.SPN chromatin is substantially unwound. The high field area tau was ~610 μs. This increase over the SPN sample is also consistent with an unwinding of the DNA. The large spectrum of relaxation times observed in the decay kinetics, even when 70 μM Mg^{++} was added, demonstrates that the sample is very flexible. Because of this flexibility, however, it is difficult to determine from the decay kinetics whether the ambiguity in extrapolating the dichroism to infinite field strength is due to a further field-induced unwinding or not.

Electron Microscopy

In the electron microscope, SPN presents its characteristic appearance, consistent with partial condensation into irregular structures (5, 8), whereas tryp.SPN appears more extended (Fig. 7). In the latter it is often possible to distinguish individual polynucleosome chains and to trace the path of the DNA along the entire length. Almost all the spacer regions in the chain are clearly defined. We have measured the lengths of more than 500 spacer segments (defined as the center-to-center spacing of adjacent nucleosomes) in randomly selected electron microscopic images of tryp.SPN. The results of this analysis are shown in Fig. 8, in which spacer lengths are represented in terms of base pairs of DNA (one base pair = 0.34 nm). The number average spacer length was found to be 95 base pairs. Two features of the data require comment: first, the distribution

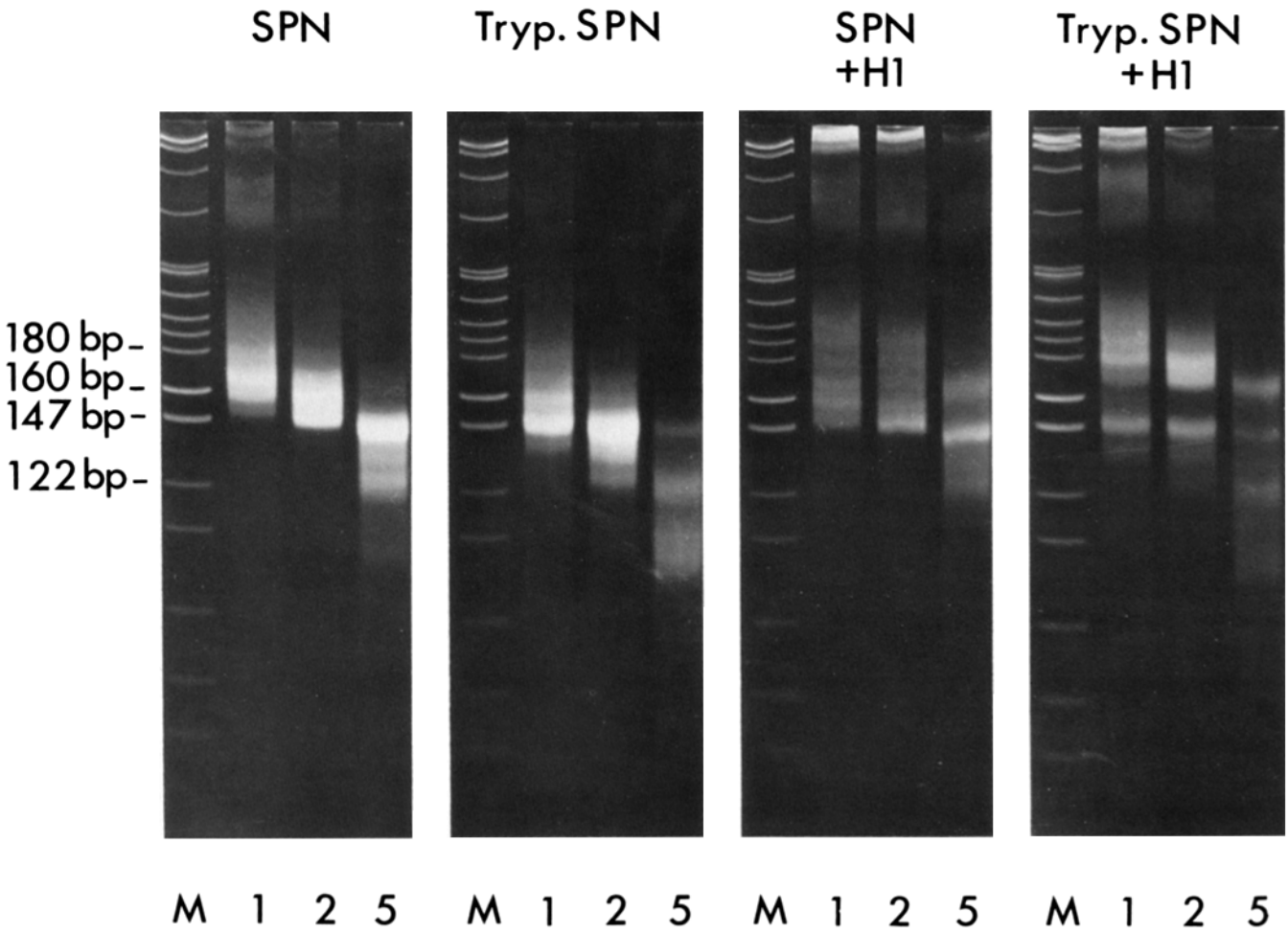

FIGURE 3 Acrylamide gel (7%) analysis of DNA isolated from micrococcal nuclease digests of *SPN*, *tryp.SPN*, and the reconstitutes derived from these chromatins. Times of digestion (min) are shown below each track. The marker (*M*) was an Hpa II digest of PBR322 DNA.

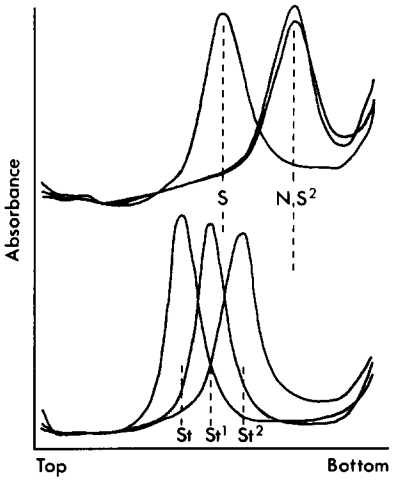

FIGURE 4 Sedimentation profiles from isokinetic sucrose gradients of stripped chromatin (*S*), native chromatin (*N*), and SPN reconstituted with two molecules of H1 per nucleosome (S^2), compared with tryp.SPN (*St*) and tryp.SPN reconstituted with one (St^1) or two (St^2) molecules of H1 per nucleosome.

of spacer lengths is skewed towards higher values and has a relatively sharp cut-off at about 125 base pairs. For chicken erythrocyte chromatin, with a repeat length of 212 base pairs, a spacer of 125 base pairs would leave only 87 base pairs of DNA associated with the core histone fragments. This, according to current estimates (29, 30), is the length of DNA required for just one full turn around the core particle. Secondly, the data suggest that the spacer lengths in tryp.SPN vary discretely

TABLE I

Sw_{20} Values of Chromatin Derived from Two Polynucleosome Fractions

Chromatin sample	Native	SPN	Tryp.SPN	SPN + H1/H5	Tryp.SPN + H1/H5
32	95^2	62^3	52^3	103^3	77^4
54	—	79^3	63^3	127^2	90^2

The chromatin sample number indicates the average fiber length (in nucleosomes) determined by measuring its DNA in an agarose gel (data not shown). The sedimentation values were the average of a number of determinations represented by the superscript. Reconstitutes were prepared at an H1 or H5 to nucleosomes ratio of 2:1, and sedimentation was in TEP_{80}.

in units of 20 base pairs. Well-defined peaks in the distribution were found at 125, 105, 85, and 65 base pairs. A 65 base-pair spacer would leave 147 base pairs of DNA in each trypsinized core particle.

The results obtained from electric dichroism bear out the structural conclusions derived from the electron microscopy. A limiting reduced dichroism of −1.05 is predicted for a chromatin of repeat length 212 base pairs with one turn of 84 base pairs in the nucleosome and 128 base pairs in the spacer {(84/212 × −0.37) + (128/212 × −1.5)} (6). This is in very satisfactory agreement with our experimental value of −0.96. The reduction in sedimentation coefficient that results from removal of the core histone tails signifies that a major confor-

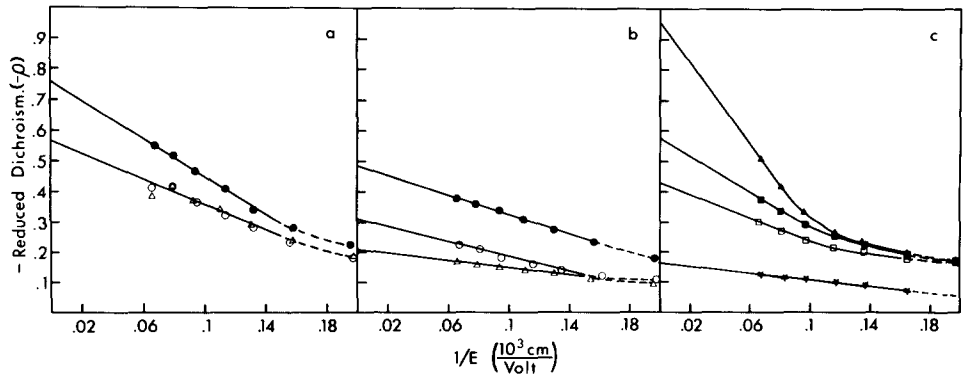

FIGURE 5 Reduced dichroism as a function of field strength for SPN (●), SPN reconstituted with one (○) or two (△) molecules of H5 per nucleosome, tryp.SPN (▲) and tryp.SPN reconstituted with H5 (■) or CH1 plus H5 before (□) and after cross-linking (★). The tryp.SPN reconstitutes were prepared at an H5 (and CH1) to nucleosome ratio of two. Measurements were made in ED buffer (*a*) or in ED buffer plus 70 μM $MgCl_2$ (*b*, *c*).

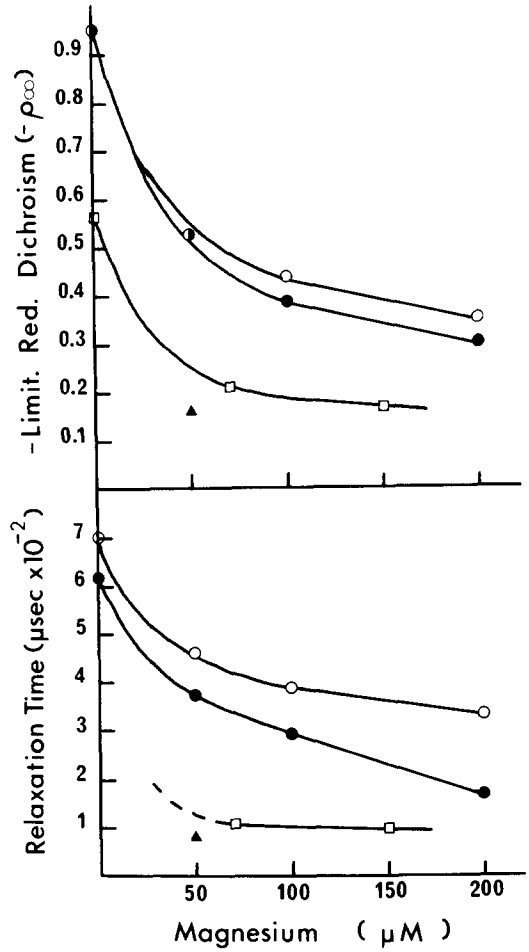

FIGURE 6 Limiting reduced dichroisms and relaxation times as a function of magnesium chloride concentration for SPN (□) and tryp.SPN (○) reconstituted with H5, and for tryp.SPN reconstituted with CH1 and H5 before (●) and after (▲) glutaraldehyde fixation. The reconstitutes were prepared at an H5 and CH1 to nucleosome ratio of two.

mational change eventuates, for the reduction in the molecular weight, which accounts for no more than 8% (12–14), and the accompanying change in partial specific volume would reduce the sedimentation coefficient by only 5%, whereas the observed reduction is nearly 20%. Thus the greatly increased rotational and translational frictional coefficients measured in solution and the increased contour length seen in the electron microscope would seem to leave no doubt that the removal of the core histone tails by trypsinization leads to a loss of structural constraints that are normally responsible for supercoiling the DNA on the core particle.

Characterization of Reconstituted Complexes Containing H1 or H5

Reconstituted chromatins were prepared with SPN and tryp.SPN using either chicken H1 or H5 linker histones. As judged by sedimentation, nuclease digestion and electron microscopy, H1- and H5-containing complexes gave essentially indistinguishable products. Only H5-containing complexes were used in the electric dichroism studies. For most studies, reconstitutes were prepared at ratios of linker histone to nucleosome of one and two. However, because chicken erythrocyte chromatin contains two molecules of linker histone per nucleosome (8, 31, 32) the bulk of the work to be described refers to complexes with this composition.

Nuclease Digestion

Complexes containing two molecules of H1 per nucleosome were prepared from tryp.SPN and SPN and were digested with micrococcal nuclease. The DNA recovered from these digests was analyzed by electrophoresis in 7% acrylamide gels. Results are shown in Fig. 3. As described earlier (8) the complex of H1 with SPN gave rise in the early stage of digestion to a progression of bands extending from 146 to 200 base pairs with an interval of about 10 base pairs. As digestion proceeded, only two bands, those of 146 and 168 base pairs, were seen to survive. Digestion of reconstituted chromatin prepared from tryp.SPN proceeded at a faster rate but also gave rise to a progression of bands of between 146 and 200 base pairs. However, here the most prominent fragments were those of 146, 178 (most intense), and 189 base pairs. After longer digestion, only the bands of 146 and 168 base pairs remained.

The protection of a DNA fragment of 168 base pairs against nuclease digestion is a characteristic of the structure of chromatin containing linker histone (8, 9, 33) and reflects the folding of two full DNA turns around the core histone octamer. Clearly, therefore, neither the loss of the core histone tails nor the associated structural changes to the core particle brought about by their absence prevents the specific attachment of H1 and the refolding of two turns of DNA about the histone octamer.

Sedimentation

When SPN is recombined with linker histone the sedimentation coefficient increases, primarily due to the formation of

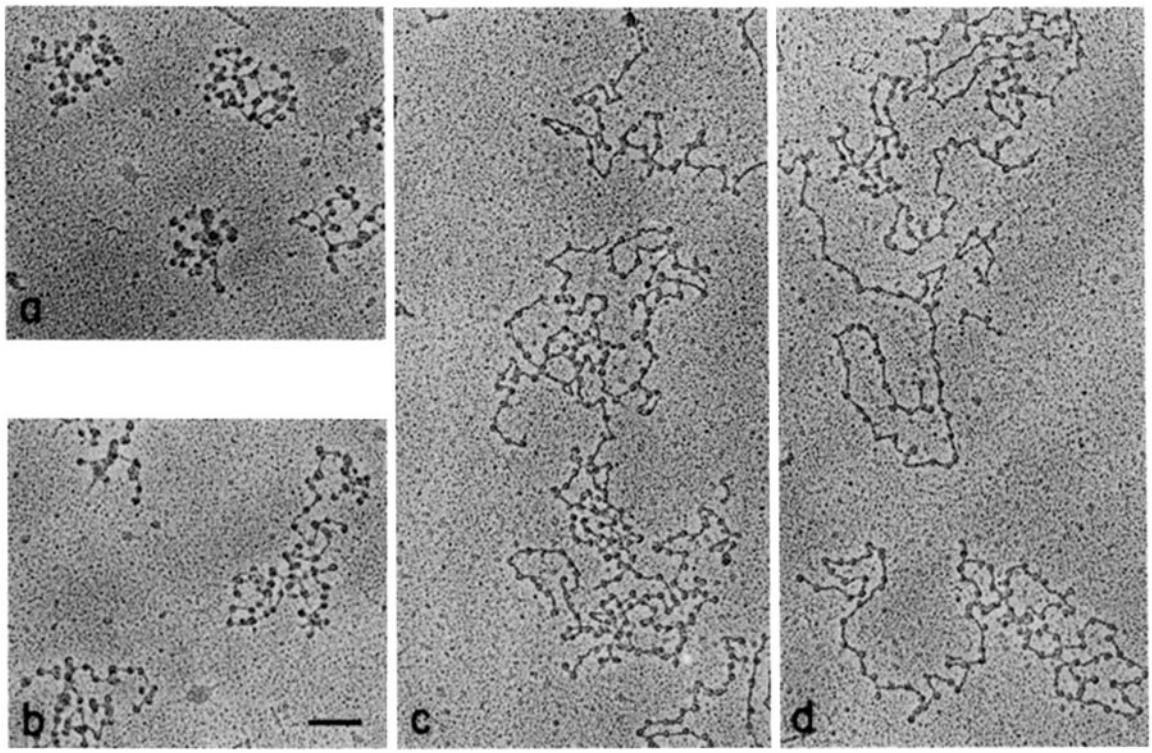

FIGURE 7 Electron micrographs of SPN (*a*, *b*) and tryp.SPN (*c*, *d*) prepared by fixation in TEP_{80}. Bar, 100 nm. × 69,000.

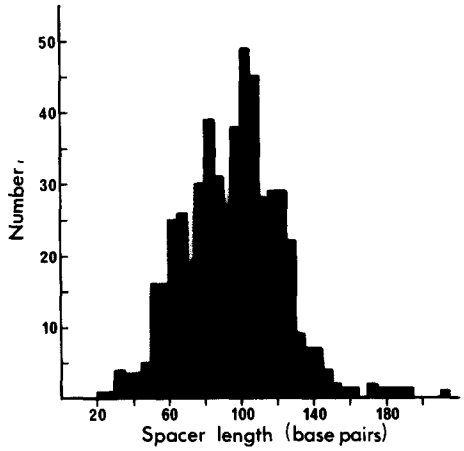

FIGURE 8 Distribution of spacer lengths measured from micrographs of tryp.SPN (see Fig. 7 *c*, *d*). Spacer lengths were measured as center-to-center distances between adjacent nucleosomes and were converted to base pairs using 1 base pair = 0.34 nm.

higher order structure. For chicken erythrocyte chromatin the sedimentation coefficient attains the value characteristic of the native state at a recombination ratio of two linker histone molecules per nucleosome (Ref. 8 and Fig. 4). Tryp.SPN shows a definite but smaller increase in sedimentation rate when recombined with one or two molecules of H1 per nucleosome (Fig. 4). Sedimentation coefficients for reconstituted chromatins derived from SPN and tryp.SPN are given in Table I. The latter are lower by ~20% on average.

In a series of experiments with ^{3}H-labeled H1, it was confirmed that all of the H1 added during reconstitution was indeed bound and sedimented with the zone of absorbing material in a sucrose gradient (data not shown). It therefore seems probable that the failure of the tryp.SPN/H1 complexes to attain the frictional properties characteristic of the native state reflects its inability to adopt a similar higher-order structure.

Electric Dichroism

Fig. 5 shows the reduced electrical dichroism as a function of field strength for complexes of SPN with one and two molecules of H5 per nucleosome, with (*b*) and without (*a*) magnesium. The extrapolated limiting reduced dichroism in the absence of magnesium was −0.58 for chromatin containing either one or two molecules of H5 per nucleosome, in excellent agreement with the value of −0.54 obtained for native chromatin in low ionic strength solution (6). Since one molecule of H5 per nucleosome suffices to generate the full amount of structural condensation, the results bear out previous models (5, 6, 9, 29, 34), whereby a single molecule of H5 is capable of closing two turns of DNA (168 base pairs) around the core histones. However, in the presence of magnesium, the complexes containing one and two molecules of H5 gave reduced dichroisms of −0.31 and −0.21, respectively (Fig. 5*b*). The latter is again in excellent agreement with our previous value of −0.20 for native chromatin (6); with lower amounts of H5 in the complex a larger negative value results.

The relaxation times obtained for these materials were 165 μs and 108 μs when they contained, respectively, one and two molecules of H5 per nucleosome. For native chromatin of the same length ($\bar{N}$ = 54 nucleosomes), a relaxation time of ~90 μs would be expected (6, 25). Furthermore, the contribution to the decay kinetics from the longest, observed relaxation time (τ_{ig}) is increasing with H5, consistent with a loss of flexibility as the chromatin compacts. For the 1 H5/nucleosome reconstitute, $\tau_{ig} \simeq 210$ μs, and contributes ~55% of the dichroism at high field strengths, as contrasted with $\tau_{ig} \simeq 2{,}000$ μs and 20–30% for the open structure. With 2 H5/nucleosome, τ_{ig} de-

creases even further to 120 μs and accounts for at least 70% of the total dichroism. Thus, as we also inferred from electron microscopy (8), it appears that two molecules of linker histone are required to encompass full condensation to the solenoid structure.

Electric dichroism results for the complex of tryp.SPN with H5 at two molecules per nucleosome are shown in Fig. 5 *c*. As for tryp.SPN, a nonlinear profile was obtained, but, even allowing for the uncertainty in the estimated extrapolated limiting value of −0.58, it is clear that the average orientation of the DNA relative to the direction of induced orientation is markedly different from that in native or reconstituted chromatins. The rather large negative value suggests the presence of an increased proportion of linear DNA, which orients itself in the direction of the applied field. That the DNA is on average in a more extended state than in the SPN complex can also be inferred from the larger relaxation time of 320 μs (as against 108 μs). The spectrum of relaxation times observed in the decay also suggests that there is only a small decrease in the flexibility of the chromatin in comparison with the data obtained in the absence of magnesium.

Electron Microscopy

Fig. 9 shows electron micrographs of SPN, tryp.SPN and their complexes with H1. It can be seen that SPN recombines with H1 to give fibers 30–34 nm. in diameter with the charac-

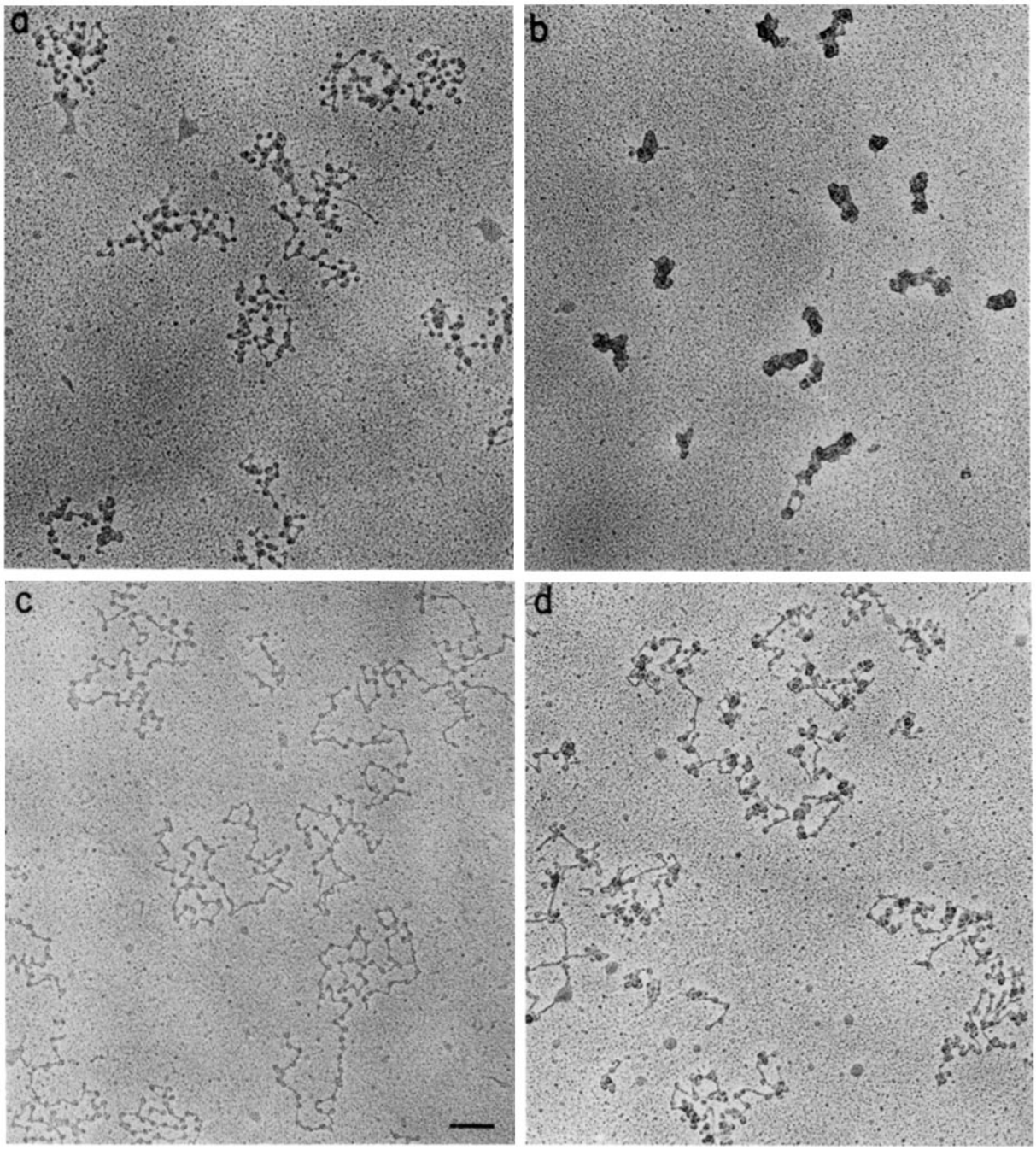

FIGURE 9 Electron micrographs of SPN (*a*) and tryp.SPN (*c*) and the reconstitutes (*b*, *d*, respectively) derived from them by addition of H1 at a ratio of two molecules per nucleosome. Bar, 100 nm. × 69,000.

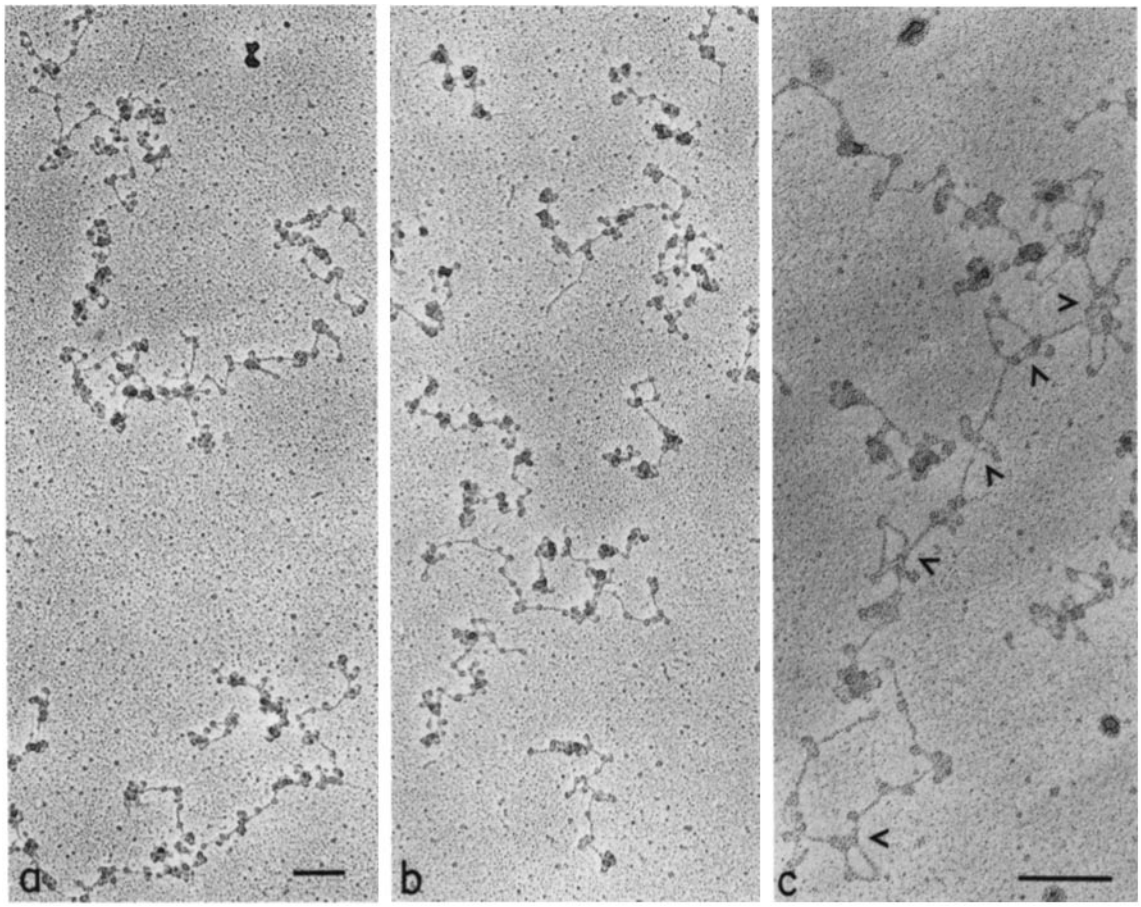

FIGURE 10 Electron micrographs of chromatins prepared by reconstitution of tryp.SPN with two molecules of H1 per nucleosome. The arrows (*c*) indicate structures described in the text. Bar, 100 nm. (*a*, *b*) × 69,000. (*c*) × 91,500.

teristic appearance of tightly packed nucleosomes (8). The complexes prepared with tryp.SPN on the other hand have a much more extended appearance (Figs. 9*d* and 10), with irregular regions rich in nucleosomes and some segments resembling condensed chromatin, some 30 nm. in diameter. Such stretches are often interrupted by segments of linear DNA or isolated nucleosomes. Condensed segments are present in most fibers but their abundance is variable. Fibers in which condensed regions are sparse often show extensive contact between nucleosomes from relatively remote parts of the chain (Fig. 10*c*, arrows). It is evident, at all events, that the absence of core histone tails drastically interferes with the capacity of H1 to induce the formation of regular higher order structures. These results confirm the conclusions based on the solution methods that the formation of the solenoid depends on the presence of the core histone tails.

Effect of Addition of CH1 to H1-Reconstituted Tryp.SPN

To clarify the structural basis for the requirement of core histone tails for higher-order structure, we have attempted to regulate the electrostatic balance within the chromatin complex in other ways. It should be noted that the tails contain no less than 30% of the positive charges of the core histone octamer (15, 30).

Large increases in counterion concentration, both univalent (Na^+, up to 230 mM) and divalent (Mg^{++}, up to 200 μM), did not materially alter the properties of the complexes between tryp.SPN and H1. On the other hand, the introduction of a 94-residue polypeptide derived from the COOH-terminal end of calf thymus H1 (9) produced a striking result. This fragment (CH1) is very basic, containing as it does 40 lysine residues but no arginines (43).

Sedimentation

Tryp.SPN was incubated first with two mole equivalents of CH1 per nucleosome and then with a similar amount of H1. The resulting complex has a sedimentation coefficient indistinguishable from that of the equivalent SPN complex (Table II). Lower proportions of H1 engendered a proportionately lower increase in sedimentation coefficient, and higher ratios rendered the complex insoluble. At 2.5 molecules per nucleosome, 75% of the complex was insoluble. In isokinetic sucrose gradients, the CH1-containing complex sedimented as a single zone which was only slightly broader than that of the complex of SPN and H1. It thus appears that the basic CH1 peptide can replace the absent core histone tails and allow the complex to enter the compact conformation characteristic of native chromatin.

Electric Dichroism

Fig. 5*c* shows the reduced electric dichroism as a function of the reciprocal field strength for the ternary complex of tryp.SPN, CH1, and H5. Again, the function is nonlinear though less markedly so than for the tryp.SPN complex. An estimate of −0.43 for the limiting reduced dichroism was obtained. The effect of CH1 on this parameter was thus modest, since the value in its absence (Fig. 5*c*) was −0.58 and that for the nativelike complex −0.21 (Fig. 5*b*). Furthermore, the mean relaxation time of 320 μs for the tryp.SPN/H5 complex was

TABLE II

Sw_{20} Values of Chromatin Derived from Two Polynucleosome Fractions

Chromatin sample	SPN + H1/H5	Tryp.SPN + H5	Tryp.SPN + CH1/H5
61	143	104	138
77	158	109	156

The chromatin sample number indicates the average fiber length (as in Table I). The reconstitutes were prepared at an H5 (and CH1) to nucleosome ratio of 2:1, and sedimentation was in TEP_{80}.

left unchanged by the addition of CH1. However, in contrast to the average area relaxation times, the decay kinetics showed a significant decrease in the observed τ_{ig}. With $[Mg^{++}]/[DNA\text{-}P] \simeq 2$, τ_{ig} for tryp.SPN/2H5 is ~1,500 μs, whereas it decreases to ~700 μs when CH1 is added and its contribution to the dichroism increases proportionately. From this, it appears that adding CH1 clearly helps in folding tryp.SPN/2H5 with Mg^{++} at low salt concentrations. In comparison with native chromatin, however, the structure is not as compact or well characterised (the limiting dichroism does not reach a limiting plateau value up to the ratio $[Mg^{++}]/[DNA\text{-}P] \simeq 6$, Fig. 6) as the SPN/2H5 reconstitute, under the same conditions.

Electron Microscopy

Fig. 11 shows the appearance of SPN reconstituted with H5 (*a–d*) and tryp.SPN associated with CH1 and H5 (*e–h*). Samples were fixed in solvents similar to those used in the solution experiments, with and without magnesium ions.

Complexes of SPN with H5 were found to have a compact structure showing some small dependence on the solvent in which they were fixed (Fig. 11 *a–d*). Whereas samples in TEP_{80} gave rise to filaments 30–34 nm in diameter, those prepared with low magnesium concentrations (70 or 140 μM) were thinner and less regular, diameters falling in the range 20–30 nm. At the highest magnesium concentration (490 μM) the filaments were found to be particularly condensed (Fig. 11 *d*). It should be noted that the SPN in this latter complex is of lower molecular weight with $\bar{N} = 49$ as against $\bar{N} = 63$ in the other preparations shown, but the relative reduction in length is greater.

The filaments formed by the ternary complex of tryp.SPN, Ch1, and H5 were, in general, indistinguishable from the above under all the conditions explored (Fig. 11 *e–h*). There was, however, always a residue, amounting to some 15% of the total, that failed to form compact structures. Such extended chains can be particularly clearly seen in preparations derived from magnesium-containing buffers (Fig. 11 *f–h*).

The evidence of electron microscopy then bears out the conclusion drawn from the sedimentation data that CH1 can replace the core histone tails. There remains the apparent conflict with the electric dichroism observations, which could be most simply explained in terms of disruption of the compact structure under the perturbing effect of the orienting field. The departure from linearity at low field strengths (Fig. 5 *c*) is consistent with such an effect. We have accordingly attempted to enhance the stability of the ternary complex by further increasing the magnesium ion concentration. This did indeed bring the limiting reduced dichroism appreciably closer to the expected value (Fig. 6), though, for the binary complex of tryp.SPN/H5, as well as for the ternary system. An increase in the concentration of the supporting electrolyte also produced an improving effect on the relaxation times, especially for the ternary complex (Fig. 6).

Much greater success was achieved by cross-linking the ternary complex with glutaraldehyde in TEP_{80}, followed by dialysis into the medium used for the electric dichroism measurements. For a sample of chain length corresponding to $\bar{N} = 63$, an extrapolated reduced dichroism of −0.16 was obtained and a relaxation time of 90 μs. The extrapolation was linear (Fig. 5 *c*) and both values approached much more closely to the expectations for a solenoid (Fig. 6).

A cross-linked tryp.SPN/H5 reconstitute, without added CH1, was also examined by electric dichroism. In contrast to the sample cross-linked with added CH1, this chromatin gave very unusual results. For a well-behaved sample, the relation between the absorbance for light polarized parallel and perpendicular to the applied electric field is that $\Delta A_{\parallel} = -\frac{1}{2}\Delta A_{\perp}$; for the cross-linked tryp.SPN/H5 reconstitute, however, both $\Delta A_{\parallel}$ and $\Delta A_{\perp}$ were small and positive ($\rho \simeq +0.02$, independent of the field strength for $E > 6$Kv/cm). This is indicative of a field-induced increase in the isotropic absorbance A. Additionally, the decay kinetics showed both positive and negative dichroism components, with relaxation times for each <200 μs. Therefore, although the sample was folded in some fashion compared with the open chromatin, it did not behave like a regular, tightly folded structure and certainly not like native chromatin. In general, it resembled some of the unusual forms observed by Lee and Crothers (Manuscript submitted for publication.).

From sedimentation and electron microscopy we are able thus to conclude that the ternary complex resembles the native structure, the CH1 substituting for the core histone tails. This is at least partly true for the electric dichroism evidence, especially after stabilization of the structure by cross-linking. Some difference, probably in the mechanical flexibility of the solenoid, evidently prevails, however.

DISCUSSION

Trypsin treatment results in the removal of the exposed terminal regions or tails of the histones that make up the octamer of the nucleosome core in chromatin. Previous work on trypsin-treated mononucleosomes has led to conflicting conclusions as to whether the core histone tails are necessary to maintain the structural integrity of these particles. On the one hand, a ten base-pair ladder of DNA fragments, extending up to 146 base pairs, is found after DNase I digestion of trypsin-treated core particles (1, 2) and, as such, demonstrates that contact between all of the DNA and the remaining protein in these particles is maintained. On the other hand, trypsin treatment of core particles also leads to a reduced sedimentation coefficient (1, 2), a change in the circular dichroism spectrum towards that of free DNA (1–3), a reduced thermal stability (2), and an increase in overall susceptibility to both micrococcal nuclease and DNase I (1, 2). Mainly on the basis of these latter observations, Lilley and Tatchell (2) concluded that removal of the core histone tails leads to a release of DNA from the confines of the core particle. Nevertheless, on the strength of the above mentioned DNase I pattern it is generally accepted that the core histone tails are not essential for maintaining the integrity of the core particle (30). From the results presented here, with H1-stripped polynucleosomes, it appears that after trypsin treatment the length of DNA in the linker segments between the cores is increased by up to 80 base pairs, leaving only one superhelical turn around the octamer. No other mechanism by which the DNA may be generated can easily be envisaged, since the DNA repeat length remains unchanged. These results should not, however, be taken as providing evidence for the role of the tails in maintaining core particle structure, for, in

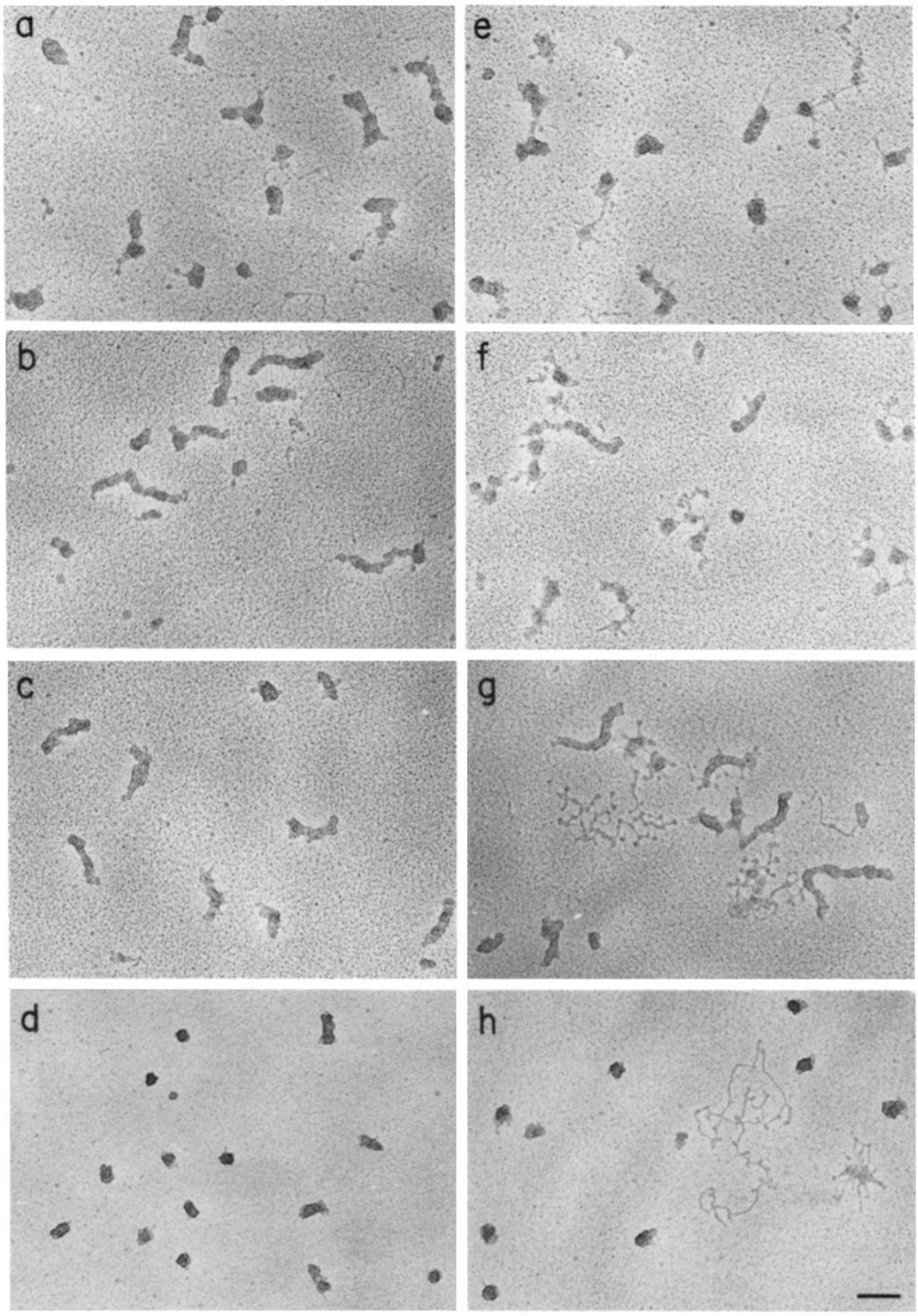

FIGURE 11 Electron micrographs of reconstituted chromatins prepared from SPN and H5 (*a–d*) and from tryp.SPN, CH1 and H5 (*e–h*). In both cases the ratio of CH1 and H5 added was two molecules per nucleosome. Samples were fixed for microscopy in TEP_{80} (*a, e*) ED buffer containing 70 μM (*b, f*), 140 μM (*c, g*) and 0.49 mM (*d, h*) $MgCl_2$. Bar, 100 nm. × 69,000.

the assays we have performed (sedimentation, electric dichroism, and electron microscopy), shearing forces will be encountered, for example during spreading for microscopy, which may substantially disrupt the structure of a long chromatin fiber (weakened by the absence of the core histone tails) but might have little effect on the stability of a core particle.

It has been proposed that the core histone tails might operate in the maintenance of higher order chromatin structure (1, 30, and Lee and Crothers, manuscript submitted for publication) and that removal or modification of these domains could alter

the stability of this fiber (39, 40). One cannot use native chromatin to test these hypotheses, because exposure to trypsin leads to the destruction of H1, which is known to be essential for survival of the higher order structure (5, 8). We have had, therefore, recourse to H1-depleted polynucleosomes which can be recombined with H1 after trypsin treatment. Our results show that the core histone tails are indeed essential for the formation of the solenoid under normal solvent conditions. By the criteria of sedimentation analysis, electric dichroism, and electron microscopy, polynucleosome chains devoid of core histone tails remain largely unfolded in the presence of H1. However, each molecule of H1 is evidently capable of stabilizing the attachment of the second turn of supercoiled DNA to the core complex, as judged by the protection of DNA segments of the corresponding length (168 base pairs) against hydrolysis by micrococcal nuclease. Since H1 restores the compact shape of the individual nucleosomes, the lack of higher order structure in the fibers is not simply a question of steric hindrance experienced in attempting to fold an unusually extended polynucleosome chain. Neither is the native structure recovered when the concentrations of either monovalent or divalent cations increased, as might be expected if the function of the tails were simply to afford electrostatic shielding of the DNA backbone.

The low specificity of the core histone tails in generating the solenoid is apparent from the ability of an extraneous basic polypeptide of unrelated sequence (CH1) to fulfill the same role. It may be remarked that two molecules of CH1 will perform the function of twelve tail segments, eight from the NH_2 termini and four from the COOH-termini of the core histones (12–14). In earlier work we showed that CH1 mixed with NGH1 (the remaining part of H1) could not replace the intact molecule of H1 in inducing higher order structure (9). The two components of H1 must presumably be covalently linked to fulfill this role. The replacement of the heterologous core histone tails with CH1 is therefore remarkable and suggests that the attachment of these domains to their trypsin-resistant complements is not an essential requirement for higher order structure. These facts underline the nonspecific nature of the mechanism of stabilization of the solenoid in the case of the core histone tails. Evidently, the highly conserved sequence of the tails, particularly of H3 and H4, (15, 30), is not directly related to their role in generating this structure.

It may be surmised that the core histone tails serve to bridge neighboring core particles in the solenoid structure. The maximum span of the fully extended tails could certainly unite nucleosomes either along the chain (55 nm apart) or across adjacent turns of the solenoid (110 nm apart). If, as has been suggested on the grounds of chemical cross-linking data, H1 links the cores along the chain (35), the second arrangement is the more likely for the core histone tails.

Whether all the tails act together in a concerted fashion, or whether different groups perform separate functions, remains to be determined. One may contemplate reconstitution of complexes of H1 with depleted polynucleosomes containing various combinations of intact and truncated core histones. As a step in this direction, we have examined reconstitutes prepared from partially trypsin-treated SPN, taking advantage of the fact that the four histones are attacked at different rates (2, 22). As Fig. 12 indicates, the loss of higher-order structure is closely correlated with the scission of H4, rather than H3, which is more rapid, or that of H2A and H2B, which is appreciably slower. Similar experiments with mononucleosomes suggested that its compact shape could be maintained by H2B alone, the last to be cleaved by trypsin (2). In themselves, these experiments are not sufficient proof for a critical role of H4 in stabilizing the higher order structure, or of H2B in maintaining the compactness of DNA in the core. The phenomena could equally well be explained by the requirement for all three histones H2A, H2B, and H4 (or certain other combinations) for the solenoid, or for at least one histone (in this case only H2B) for the nucleosome structure. Clearly, other permutations have to be tested.

A nucleosome in which the core histone tails are missing or ineffective would not only be structurally aberrant in itself but might be expected to give rise to a dislocation in the solenoid. If such anomalies occurred in vivo, either the elongated spacer region or the locally relaxed packing of the nucleosomes might be expected to give preferential access to enzymes, such as RNA polymerase; either initiation of transcription or chain propagation could thereby be facilitated. Studies in this laboratory (Hannon and Gould, unpublished observations) have indeed shown that after tryptic removal of the core histone tails from SPN the number of initiation sites for *E. coli* RNA polymerase is doubled.

There is, of course, no evidence that proteolysis of tails in fact occurs in vivo although proteases associated with chromatin, and specific for certain of the core histones, have been identified (36, 37). As the limited evidence obtained so far is consistent with the possibility that the loss of higher order structure might be uniquely associated with only one tail, perhaps that of H4, these enzymes could play a significant role in modulating chromatin structure. A more compelling model for the local reversal of DNA condensation, based on a different mechanism for the annihilation of positive charges, namely acetylation of ϵ-amino groups, has been proposed (39, 40). A correlation between acetylation and transcriptional activity in vivo is well documented (see references 16 and 17 for review). Moreover, hyperacetylated chromatin (38), from cells treated with sodium butyrate, an inhibitor of deacetylases, is preferentially degraded by DNase I (18, 19) in regions rich in multiacetylated core histones (39, 40). The implication is that chromatin structure is in some manner rendered more accessible to the nuclease by acetylation. The acetylation sites, of which there are four on histones H3, H4, and H2B and one on H2A, are all known to be located in the amino terminal trypsin-labile tails (15, 16). Thus, 26 of the 42 lysine residues present in the core histone tails of each nucleosome are prone to acetylation. Such a potentially large change in the charge balance could lead to a major pertubation of the chromatin structure and thus allow access to molecules such as DNase I or RNA polymerase. The total removal of the core histone tails by proteolysis with trypsin in vitro may be tentatively regarded as an analogue for this intracellular phenomenon.

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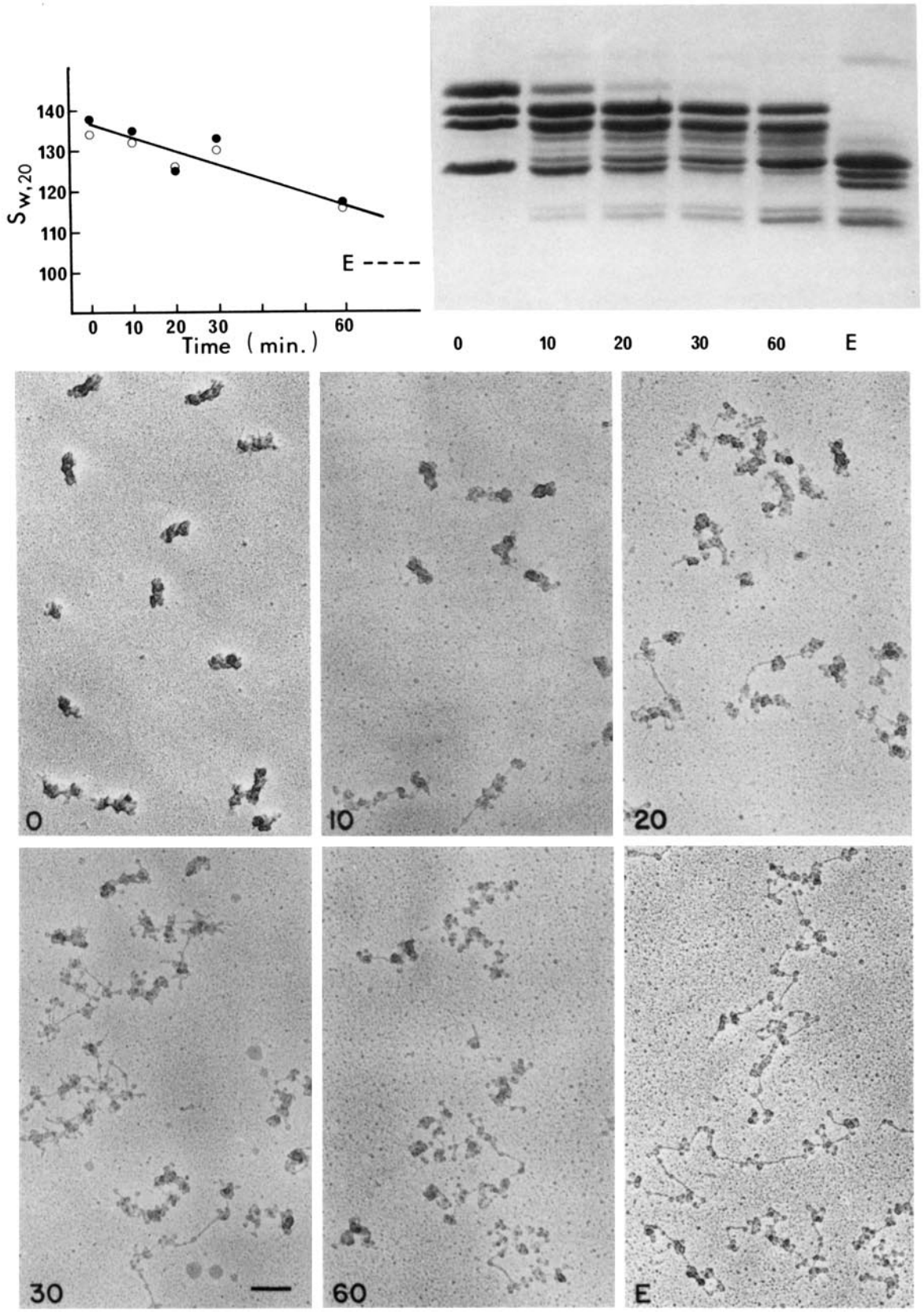

FIGURE 12 Sedimentation coefficients (upper left) and electron micrographs (bottom) of reconstitutes prepared from a series of SPN samples that had been digested with trypsin (0.2 μg/ml.) for different times. The extent of trypsin degradation achieved in each sample is shown by the 18% SDS acrylamide gel analysis (top right). Times of digestion (min) are shown below each track or micrograph. The extensive digest (*E*) was prepared under standard conditions (Materials and Methods). Reconstitutes were prepared at a ratio of two H1 molecules per nucleosome. Sedimentation and fixation for microscopy was in TEP_{80}. Bar in the micrographs, 100 nm. × 69,000.

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