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Histone H5 Can Increase the Internucleosome Spacing in Dinucleosomes to Nativelike Values†

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ABSTRACT: Chicken erythrocyte chromatin was assembled with inner histones at about 60% of the ratio found in vivo and subsequently incubated with histone H5 (or H1 + H5) in a solution containing 0.1 M NaCl and poly(glutamic acid). Micrococcal nuclease digestion produced dinucleosomes of 360–390 base pair (bp) DNA content, similar to those from native chromatin and contrasting with the 270–280 bp species found in material incubated without H5. On sucrose gradients a dinucleosome sedimenting at 16 S containing 360 bp DNA was isolated. Removal of H1 + H5 after reconstitution did not change these results; H5 thus can induce rearrangements of nucleosome cores with respect to their neighbors. The results are interpreted as an H5-induced "sliding apart" of histone octamers, complementary to the "sliding together" found in native chromatin after removal of H1 + H5.

Histone H1 has long been implicated in the condensation of chromatin (Bradbury et al., 1973; Billett & Barry, 1974). More recently, detailed studies of the ionic strength dependence of the condensation of chromatin have shown that H1 is required for chromatin to condense to a high degree and with structural regularity (Renz et al., 1977; Thoma et al., 1979; Strätling, 1979; Ruiz-Carrillo et al., 1980). It has also been shown that when chromatin is stripped of H1 by procedures that do not disturb the nucleosome spacing, many of the properties of native chromatin can be reestablished by adding H1 back (P. P. Nelson et al., 1979; Allan et al., 1980; Thoma & Koller, 1981).

Whereas the role of H1 in chromatin condensation is fairly well established, the relationship between H1 and the nucleosome spacing is not. The native nucleosome spacing has been achieved in vitro only for unfractionated cell homogenates from *Xenopus* eggs (Laskey et al., 1977; Laskey & Earnshaw, 1980) or *Drosophila* embryos (T. Nelson et al., 1979). These extracts contain endogenous histones, many other proteins, and a variety of enzymatic activities. The "active ingredients" for nucleosome spacing have not been identified. Because of the tissue and species specific variability of H1 and the apparent interaction of H1 with linker DNA, it has been suggested that H1 may specify the nucleosome repeat length in chromatin (Morris, 1976a; Noll, 1976; Compton et al., 1976). However, for chromatin reconstituted in vitro from purified histones and DNA, it has been found that nucleosomes pack closely together with repeat lengths much shorter than those generated in vivo (Yaneva et al., 1976; Steinmetz et al., 1978; Thomas & Butler, 1978; Spadafora et al., 1978; Fulmer & Fasman, 1979; Ruiz-Carrillo et al., 1979; Noll et al., 1980). Similarly, when H1 is removed from chromatin, nucleosomes tend to slide together under conditions where the nucleosome spacing is stable in native chromatin (Spadafora et al., 1979; Weischet,

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1979). Presently, it is not known whether H1 is actively involved in setting the internucleosome spacing or H1 merely stabilizes it.

Attempts to reconstitute chromatin from purified histones and DNA have been plagued by the tendency of the nucleoprotein to precipitate in the presence of H1, close to physiological ionic strength. Unfortunately, there are several reasons to believe that salt concentrations in the physiological range are required for the correct interactions between H1 and nucleosomes to occur. First, the degree of condensation of chromatin is very ionic strength dependent. At low ionic strength, chromatin is an extended filament of nucleosomes, whereas near physiological ionic strength, chromatin exists as thick 250-Å diameter fibers (Renz et al., 1977; Thoma et al., 1979; Ruiz-Carrillo et al., 1980). Second, the correct addition of H1 to H1-stripped chromatin requires the presence of salt (Allan et al., 1980). Third, nucleosomes in H1-depleted chromatin can slide to some extent at near physiological salt concentrations (at 37 °C) but not at low ionic strengths (Spadafora et al., 1979; Weischet, 1979). Therefore, if H1 is added to reassembled chromatin at very low ionic strength, the correct interactions will very likely not be able to occur, whereas if H1 is added at physiological ionic strength, extensive nucleoprotein aggregation occurs. Clearly, this problem should be considered when reconstitution experiments involving H1 are interpreted.

We have found that the H1-induced insolubility problem at physiological ionic strength can be completely overcome by including 2 mg of poly(glutamic acid)/mL in the reacton mixture. Poly(glutamic acid) greatly facilitates the assembly of chromatin from DNA and core histones at physiological ionic strength (Stein et al., 1979). Also, in solutions containing poly(glutamic acid), H1 + H5 interacts with nucleosomes to increase the supercoiling of nucleosomal DNA in reconstituted chromatin (Stein, 1980); in the absence of poly(glutamic acid), the chromatin aggregated, and no effect of H1 + H5 was observed. In this study, we report and examine an effect of H5 (or H1 + H5) on the internucleosome spacing in a sample consisting of, predominantly, reconstituted dinucleosomes (those nucleosomes close to each other on a long fragment of DNA).

Materials and Methods

Micrococcal nuclease (Worthington) was dissolved in water at 10 units/μL and stored frozen. A 10 mg/mL stock solution of poly(glutamic acid) of average molecular weight 100 000 (Miles), in 25 mM Tris-HCl,[1] pH 7.0, was prepared by neutralization with sodium hydroxide. Hydroxylapatite (DNA grade) was purchased from Bio-Rad. φX174 RF DNA digested with *Hin*cII was purchased from New England Biolabs. Chicken blood was purchased from Pel-Freez.

Preparative Procedures. Chicken erythrocyte nuclei were prepared as described by Hymer & Kuff (1964). For preparation of DNA, nuclei were first digested at 37 °C for 16 h with 0.2 mg of proteinase K (Boehringer)/mL in 10 mM EDTA and 0.1% $NaDodSO_4$. The sample was then adjusted to 1% $NaDodSO_4$, 0.1 M Tris-HCl, pH 8, and 10 mM EDTA, extracted 4 times with phenol saturated with the same buffer and once with chloroform/isoamyl alcohol (24/1), ethanol precipitated, and dialyzed extensively against 0.1 M NaCl, 20 mM Tris-HCl, pH 7.2, and 0.2 mM EDTA. In some experiments mildly sonicated DNA was used. Core histones were prepared form nuclei by modification of the method of Jamaluddin et al. (1979). Nuclei were equilibrated with 0.7 M NaCl and 50 mM sodium phosphate, pH 7.0, and mixed with a suspension of hydroxylapatite (5 g/25 mg of DNA). The suspension containing lysed nuclei was washed 6 times with the same buffer, using low-speed centrifugations, to remove H1 and H5. Core histones were extracted by using 2.5 M NaCl and 50 mM sodium phosphate and were concentrated by ultrafiltration in an Amicon cell by using a YM10 membrane. For preparation of H5 containing about 10% H1 and trace amounts of H2A and H2B, nuclei were first washed 4 times with 0.5 M NaCl, 10 mM Tris-HCl, pH 8, and 1 mM EDTA to selectively remove H1 histones (Johns & Diggle, 1969), then hydroxylapatite was added, and H5 (contaminated with some residual H1) was extracted by using 0.7 M NaCl and 50 mM sodium phosphate. Extinction coefficients ($A_{280}^{1\%}$) used for core histones (Stein & Page, 1980) and H5 (Johns, 1971), respectively, were 4.4 and 2.2. Alternatively, H1 + H5 was extracted from 0.3 M NaCl washed nuclei with 5% perchloric acid. H5 or H1 + H5 was concentrated as described above and dialyzed against 0.1 M NaCl and 20 mM Tris-HCl, pH 7.2.

Reconstitution Reactions. Chromatin was reconstituted from DNA and core histones by salt-gradient dialysis. Histones and DNA were mixed in 2.0 M NaCl, 20 mM Tris-HCl, pH 7.2, and 0.2 mM EDTA and dialyzed against 1.0 M NaCl for 1 h, 0.8 M for 4 h, 0.6 M for 12 h, and 0.0 M for 2 h at room temperature (Simpson & Künzler, 1979). Phenylmethanesulfonyl fluoride in Me_2SO was added to the first dialysis solution to a concentration of 0.2 mM. For incubations with H5, this chromatin was adjusted to either 2.0 or 0.5 mg of poly(glutamic acid)/mL and dialyzed against 0.1 M NaCl, 20 mM Tris-HCl, pH 7.2, and 0.2 mM EDTA. Interestingly, chromatin thus prepared, using high molecular weight DNA, is completely soluble at 0.1 M ionic strength in the presence of poly(glutamic acid), but only partially soluble in the absence of this component. Histone H5 (or H1 + H5) was mixed with an 8-fold weight excess of poly(glutamic acid), each component in 0.1 M NaCl and 20 mM Tris-HCl, pH 7.2. The salt-extracted H5 was completely soluble instantaneously, whereas the acid-extracted H1 + H5 required several hours to completely go into solution. A concentrated H5 (or H1 + H5) containing solution, generally greater than 2 mg/mL in poly(glutamic acid), was added to about a 10-fold larger volume of chromatin reconstituted with core histone (100 μg DNA/mL, 2.0 or 0.5 mg of poly(glutamic acid)/mL), and the sample was incubated 3 h at 37 °C. In control samples not containing H5 (or H1 + H5) a blank solution was added to the chromatin so that the concentrations of poly(glutamic acid) and other components were identical in both samples.

In one experiment, a 10-fold molar excess of salt-extracted H5 was added to reconstituted chromatin in a solution containing 2.0 mg of poly(glutamic acid)/mL. In another experiment a 1.5-fold molar excess of acid-extracted H1 + H5 was added to reconstituted chromatin in a solution containing 0.5 mg of poly(glutamic acid)/mL.

Removal of H1 and H5 from Chromatin. For complete removal of H1 and H5 from either native or reconstituted (using 1.5 mol of H1 + H5/mol of core octamer) chromatin, 1 mL of the sample in 20 mM Tris-HCl, pH 7.2, 0.2 mM EDTA, and 0.5–2.0 mg/mL poly(glutamic acid) was layered onto a tube containing about 10 mL of 5% sucrose, 20 mM Tris-HCl, pH 7.2, and 0.2 mM EDTA and a 1-mL 50% sucrose cushion and centrifuged 8 h at 40 000 rpm, 20 °C, with

[1] Abbreviations: bp, base pairs; PMSF, phenylmethanesulfonyl fluoride; $NaDodSO_4$, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetate; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; Me_2SO, dimethyl sulfoxide.

a Beckman SW41 Ti rotor. Approximately 70% of the input DNA was typically recovered in the pellet. This procedure also removes the poly(glutamic acid) from the sample. Poly(glutamic acid) coextracts with DNA in preparing samples for electrophoresis and can be detected on gels by staining with Stains-All. Samples electrophoresed directly exhibited an intense bluish-purple staining with upper cutoff in the position of approximately 200 base pair DNA. No detectable staining in this region was observed with DNA extracted from pelleted chromatin. Poly(glutamic acid) does not stain with ethidium bromide under UV illumination.

Other Procedures. For micrococcal nuclease digestions, chromatin samples, after incubation in the presence or absence of H5, were dialyzed against 20 mM Tris-HCl, pH 7.2, and 0.2 mM EDTA, equilibrated at 37 °C, and adjusted to 0.6 mM $CaCl_2$. Digestions were stopped by adjusting aliquots of the sample to 0.1% $NaDodSO_4$ and 10 mM EDTA, and samples were incubated 2 h or more at 37 °C with 0.2 mg/mL proteinase K. Samples were then adjusted to 1% $NaDodSO_4$, 0.1 M Tris-HCl, pH 8.0, and 10 mM EDTA and extracted twice with phenol (saturated with the same buffer) and once with chloroform/isoamyl alcohol (24/1). Samples were next adjusted to 0.3 M sodium acetate, precipitated with 2 volumes of ethanol, dried under vacuum, and dissolved in sample buffer for electrophoresis. DNA samples were electrophoresed on either 2.5% agarose gels or on 5% polyacrylamide gels by using the Tris/borate buffer system (Peacock & Dingman, 1967). Gels were stained with 3 μg of ethidium bromide/mL for 15 min, soaked in water overnight, and photographed under UV illumination by using a Kodak No. 21 Wratten filter. Negatives were scanned on an E-C Apparatus densitometer.

In order to trim the tails from nucleosome oligomers, we digested the sample with BAL-31 exonuclease (Bethesda Research Labs) in a solution containing 20 mM Tris-HCl, pH 7.0, 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 2 mg of poly(glutamic acid)/mL, at room temperature. Nucleosome oligomers were produced by digesting H5- (and H1-) stripped chromatin (0.5 A_{260} unit/mL) with 0.01 unit of micrococcal nuclease/μg of DNA in 20 mM Tris-HCl, pH 7.0, 0.2 mM EDTA, and 0.6 mM in $CaCl_2$, at 37 °C for 10 min; the sample was then chilled on ice and adjusted to the BAL-31 digestion conditions. The presence of the poly(glutamic acid) inhibited micrococcal nuclease activity about 40-fold in addition to the reduction in activity by about a factor of 5 due to lowering the temperature. Under these conditions, negligible additional micrococcal nuclease digestion occurred during the BAL-31 digestion (5 min).

Isokinetic sucrose gradients (McCarty et al., 1974) were prepared for particle densities of 1.51 g/cm^3 at 4 °C, C_m = 10%. Samples were centrifuged at 40 000 rpm at 5 °C for 16 h. Gradients were emptied by pumping from the bottom of the tube through a Pharmacia UV-1 monitor, or fractions were collected and absorbance was measured by using a Cary 219 spectrophotometer. Sedimentation coefficients were determined from the peak positions of native chromatin oligomers run in parallel with samples on separate tubes; values of 11 S and 16 S were used for mono- and dinucleosomes, respectively (Noll, 1974). To analyze the DNA from sucrose gradient fractions by electrophoresis, we first precipitated the nucleoprotein in fractions with 4 volumes of ethanol after adding bovine serum albumin to a concentration of 50 μg/mL.

Histones were analyzed on 18% polyacrylamide gels containing $NaDodSO_4$ with a 3% stacking gel (Le Stourgeon & Rusch, 1973). Gels were scanned by using an E-C Apparatus densitometer.

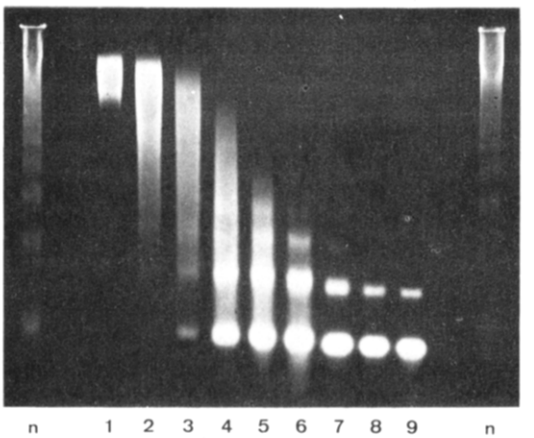

FIGURE 1: Micrococcal nuclease digestion of chromatin reconstituted from chicken erythrocyte DNA and core histones. Chromatin was reconstituted form 0.6 μg of core histones/μg of DNA and digested with 0.03 unit of enzyme/μg of DNA (at 3.0 A_{260} units/mL) for the times stated. DNA was extracted and electrophoresed on a 2.5% agarose gel, about 10 μg/lane. In lanes 1–9, digestion times were 0, 0.5, 1, 2, 4, 6, 10, 20, and 30 min. Lanes labeled n are a digest of chicken erythrocyte nuclei.

Results

Incubation of Reconstituted Chromatin with H5 and Poly(glutamic acid) Produces Nativelike Nuclease-Resistant DNA Fragments. Reconstitution of chromatin from DNA and core histones at a histone to DNA ratio of 0.6 (w/w) produces predominantly randomly spaced nucleosomes interspersed with free DNA gaps. Few clusters exist containing more than two or three closely spaced histone octamers (Noll et al., 1980). When such chromatin is digested to increasing extents with micrococcal nuclease, the DNA fragments produced appear as in Figure 1. At early times in the digestion nonspecific lengths of DNA are produced, consistent with the existence of large regions of free DNA. Several bands that are multiples of about 150 base pairs, reflecting the existence of closely packed nucleosomes, can also be discerned, superimposed upon the high background. At later times, the background diminishes and higher order oligomers get digested away. Remaining dinucleosome DNA fragments become shorter due to an exonuclease activity associated with micrococcal nuclease (Noll & Kornberg, 1977; Weischet et al., 1979; Noll et al., 1980).

When this reconstituted chromatin is incubated with poly(glutamic acid) in a solution containing 0.1 M NaCl and then digested at low ionic strength [in the presence of poly(glutamic acid)], digestion patterns similar to those in Figure 1 are produced (Figure 2A, lanes 1–4). However, when H5 is included in the incubation mixture, longer nativelike bands become apparent (lanes 5–8). Densitometer scans of lanes 1, 5, and n of the photographic negative of this gel are shown in Figure 2B. Nativelike bands up to trimers can be discerned in lane 5. At the lowest extent of digestion (lane 5), the monomer and dimer bands are somewhat longer than those resulting from the digest of nuclei shown (lanes n) and are similar to the bands produced from more lightly digested nuclei. Figure 2C shows the DNA fragments from nuclei digested to several extents along with size standards. Note that the midpoint of the dimer band decreases from 425 to 350 base pairs over the course of the digestion. The repeat length in chicken erythrocyte chromatin has been reported to be 212 base pairs (Morris, 1976b). In Figure 2B, backgrounds are high, but they are similar for the samples in lanes 1–4 and lanes 5–8, respectively. Thus, the background in the reconstitute containing H5 (lanes 5–8) probably cannot be attributed

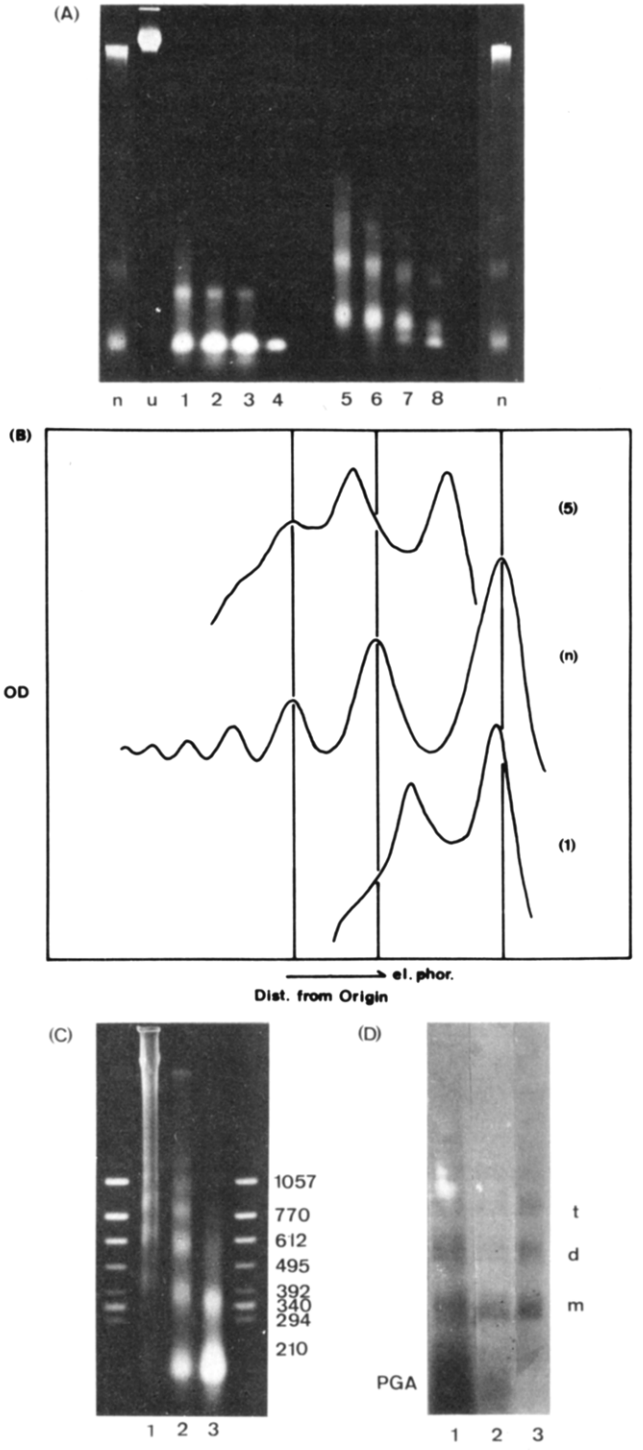

FIGURE 2: Micrococcal nuclease digestions of reconstituted chromatin after incubation in a poly(glutamic acid) solution without or with H5 on 2.5% agarose gel. Samples were digested with 4 units of enzyme/μg of DNA for 1, 2, 4, and 8 min. Lanes 1–4 are digests of a sample incubated in the absence of H5; lanes 5–8 are digests of a sample incubated in the presence of H5. Lanes labeled n are from a digest of nuclei; lane u is an undigested sample. (B) Scans of lanes 1, 5, and n of the negative of the photograph in (A). (C) Agarose gel (2.5%) of DNA from chicken erythrocyte nuclei digested in increasing extents, lanes 1–3; the size markers are a *Hin*cII digest of ϕX174 RF DNA. (D) Agarose gel (1.5%) of DNA from chicken erythrocyte nuclei, digested with micrococcal nuclease to a similar extent in the presence of varying amounts of poly(glutamic acid) (PGA). Staining with Stains-All (Eastman). Lanes 1 and 2, 6:1 and 2:1 PGA to DNA ratio, respectively; lane 3, no PGA added. "m", "d", and "t", positions of mono-, di-, and trinucleosome bands. "PGA", typical poly(glutamic acid) bands (color is more purple than DNA bands); the position of these bands varied somewhat depending on the gel system and run conditions but was always below the 200 bp DNA position on the gel.

solely to the effects of H5. The concentrations of poly(glutamic acid) and all other components except H5 were the same in both samples.

The major effect of poly(glutamic acid) alone is on the rate of digestion; about 40 times more enzyme (or longer time) is required to achieve the same extent of digestion. This is also true for purified DNA and native chromatin (not shown). Moreover, for native chromatin, essentially identical band patterns are produced for samples incubated and digested in the presence or absence of poly(glutamic acid) (Figure 2D). A minor effect of poly(glutamic acid) on micrococcal nuclease digests of chromatin is that slightly higher backgrounds result at extensive levels of digestion (compare Figure 2A, lanes 1–4, with Figure 1). This effect could arise simply from a slight decrease in the rate of digestion of linker DNA relative to core DNA for the [poly(glutamic acid)] inhibited nuclease. Poly(glutamic acid) does not induce irreversible alterations in the chromatin structure (unpublished observations, and see below).

Presence of H5 (or H1) during the Digestion Is Not Necessary for the Production of a Nuclease-Resistant Nativelike Dimer. A trivial explanation for the appearance of a nativelike dimer band upon nuclease digestion of the reassembled chromatin would be that several molecules of H5 bind to pairs of closely packed histone octamers on the DNA in such a way as to protect additional DNA at the ends from digestion. In order to exclude this possibility, we removed H5 (and H1) from the reassembled chromatin by a method that does not change the repeat length of native chromatin. If the newly observed digestion barriers were simply the consequence of H5 (and H1) coating the DNA ends, removal of H5 and H1 should completely abolish them; the nuclease digestion pattern should again look like the one of control material not exposed to H5. On the other hand, if incubation with H5 increased the internucleosome spacing, the nuclease digestion pattern should look like that of H5- (and H1-) stripped native chromatin.

(1) Digestion of H5- (and H1-) Stripped Native Chromatin. Attempts to selectively remove H5 and H1 from chicken erythrocyte chromatin by anionic resin treatment (Bolund & Johns, 1973; Modak et al., 1980) proved unsuccessful at chromatin concentrations below 2 A_{260} units/mL; usually most of the inner histones were removed from the DNA together with H5 and H1. We found, however, that chicken erythrocyte chromatin, mixed with sufficient poly(glutamic acid) at low ionic strength, could be depleted of H5 and H1 at low chromatin concentrations by sedimentation through 5% sucrose (see Materials and Methods). Removal of H5 and H1 by this method does not alter the nucleosome spacing, as shown in Figure 3A. Note that the monomer band is quite long, containing DNA longer than 210 base pairs at early stages of digestion, and that it splits into two bands as core particles appear later in the digestion. This type of nuclease digestion pattern is highly characteristic of H1-depleted chromatin, when depletion is performed by methods that do not disturb the nucleosome spacing. Very similar patterns have been reported for rat liver chromatin (Spadafora et al., 1979) by using the tRNA H1-depletion method (Iliyn et al., 1971) and for calf thymus nuclei (Weischet et al., 1979) by using the low pH method of H1 depletion (Cole et al., 1978).

We next performed an experiment to verify that the 210 base pair (average size) band arises from a long mononucleosome rather than some type of unusually short dinucleosome, generated during the H5- (and H1-) stripping procedure. "Compact dinucleosomes" containing DNA lengths

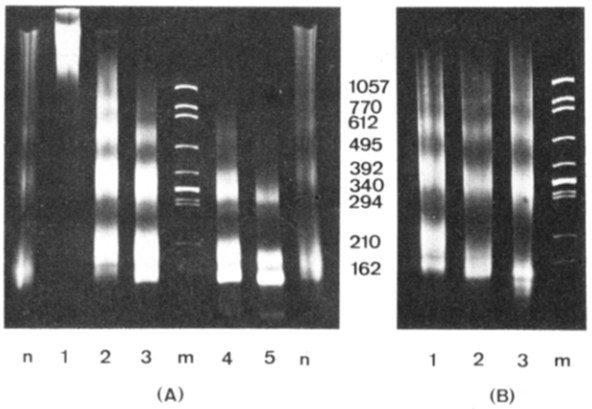

FIGURE 3: Digests of (H1 and H5)-stripped native chromatin. (A) Polyacrylamide gel (5%) of DNA fragments from stripped chromatin digested with 0.2 unit of micrococcal nuclease/μg of DNA for 0, 0.5, 1, 2, and 5 min (lanes 1–5). Lanes labeled n are from a digest of nuclei, and lane m is a *Hin*cII digest of ϕX174 RF DNA. (B) Polyacrylamide gel (5%) of DNA from nucleosome oligomers trimmed with BAL-31 exonuclease. The sample was digested with 2 units of enzyme/μg of DNA for 0, 2, and 5 min, (lanes 1–3). Lane m is a *Hin*cII digest of ϕX174 RF DNA.

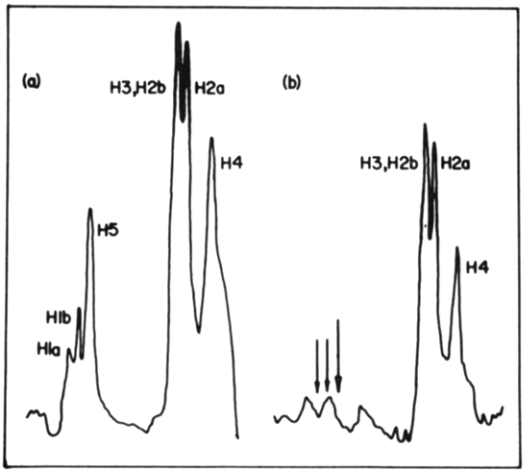

FIGURE 4: Histones before and after H1 + H5 depletion. Scans of a histone gel; reconstituted material was precipitated with 4 volumes of ethanol. The pellets were dissolved in sample buffer, boiled, and applied to the gel. (a) Material reconstituted with full complement of histones. The ratio of peak areas of the H1 + H5 peak to the core histone peak is 0.27. This corresponds to an H1 + H5 to core histone–octamer ratio of 1.4, consistent with the input ratio of 1.5 chosen for reconstitution. (b) Material after depletion of H1 + H5. Vertical arrows indicate positions of the two H1 (smaller arrows) and the H5 (large arrow). The difference in core histone peaks between (a) and (b) reflects differences in total nucleoprotein precipitated. The ripples in (b) are not above background if compared to scans over unstained parts of the gel.

as short as 240 base pairs have been observed in extensively digested H1-stripped (by a method using 0.6 M NaCl) chromatin (Klevan & Crothers, 1977). The existence of such particles would interfere with our analysis. Long-tailed mononucleosomes should be readily distinguishable from short "nibbled-down" compact dinucleosomes by their sensitivity to exonuclease. Therefore, we first digested H5- (and H1-) stripped chromatin with micrococcal nuclease to the extent shown in Figure 3B, lane 1 (approximately the same as in Figure 3A, lane 3). Then, under condtions where micrococcal nuclease was inhibited (see Materials and Methods), the chromatin oligomers were digested lightly with BAL-31 exonuclease (Figure 3B, lanes 2 and 3). Figure 3B shows that the 210 base pair band is highly sensitive to the exonuclease (compared to the dinucleosome band) and that it is readily converted into core particle and shorter DNA lengths. This indicates that the 210 base pair band arises from mononucleosome-like particles containing tails of DNA, easily accessible to exonuclease degradation. The result of this experiment excludes the presence of nuclease-resistant compact dinucleosomes in our sample.

(2) *Digestion of H5- (and H1-) Stripped Reassembled Chromatin.* With this technique for removing H1 and H5, we removed H1 and H5 from chromatin reassembled from 0.5 mg of core histones/mg of DNA and 1.5 mol of H1 + H5/mol of core histone octamer. Densitometer scans of an $NaDodSO_4$ gel showing the histones present before and after H1 and H5 removal are shown in Figure 4. The (H1 and H5)-stripped reassembled chromatin was then digested with micrococcal nuclease, and the nucleoprotein particles were fractionated on a sucrose gradient to separate mono- and dinucleosomes and to remove a continuum of free DNA fragments that electrophorese in the range of DNA fragments extracted from nucleosome oligomers. Sucrose gradient fractions were collected, and the DNA from fractions ranging from 10 to 20 S was extracted and electrophoresed. The whole experiment was also performed on a control sample to which H1 + H5 was not added, although the concentrations of poly(glutamic acid) and all other components were identical.

Figure 5a shows the UV absorption profile of the sucrose gradient. The 11S monunucleosome peak (fractions E and D) and the 16S dinucleosome peak (fractions B and C) cosediment with those of the control and of native chicken erythrocyte chromatin (not shown). Figure 5b shows that the dimer fraction DNA (lane B) has a nativelike length of 360 base pairs for the sample incubated wth H1 + H5, whereas the dimer fraction DNA for the control sample incubated without H1 + H5 (lane R) has a considerably shorter length of 280 base pairs. Morevoer, the DNA fragments produced from (H5 and H1)-stripped reassembled chromatin are very similar to those from (H5 and H1)-stripped native chromatin at comparable extents of digestion (compare with Figure 3A). In fact, fraction A reveals a small amount of a nativelike trimer. Additionally, the 210 base pair particle cosediments with core particles (fractions D and E), and it is readily digested to a core particle with BAL-31 exonuclease (not shown), confirming that this particle is a mononucleosome. Therefore, the persistence of the nativelike dinucleosome band upon digestion after removal of H1 and H5 and the absence of 280 base pair band (close packed dimer) indicate that H5 (or H1) caused closely packed dinucleosomes to move apart.

Discussion

We have presented evidence that H5 in the presence of poly(glutamic acid) can move histone octamers apart; closely packed dinucleosomes become nativelike dinucleosomes. This effect complements the sliding together that occurs upon removal of H1 from native chromatin (Spadafora et al., 1979; Weischet et al., 1979).

The core histone to DNA ratio chosen for our experiments was such that few close packed octamers in groups of more than two should be expected. This situation is optimal for studying effects of H5 on the relative spacing of octamers in nucleosomes. At higher inner histone to DNA ratios (greater than 0.6g/g of DNA), this experiment would become much more complex. Octamer crowding effects would necessarily come into play, and dimers would also be produced from the digestion of higher order oligomers. When we performed experiments similar to those described here, but at higher core histone to DNA ratios, we observed, upon incubation with H5, a mixture of nativelike and close packed dimers in addition

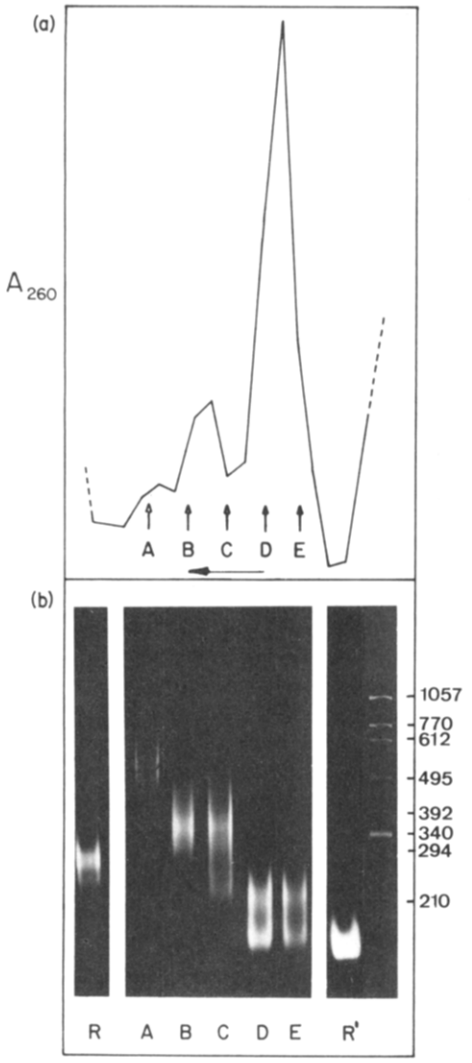

FIGURE 5: Fractionation of reconstituted chromatin depleted of H1 + H5. Histone content before and after depletion is shown in Figure 4. Input core histone to DNA ratio was 0.55. Digestion was with 0.06 unit of enzyme/μg of DNA for 1.3 min. (a) Absorption profile of sucrose gradient. Direction of sedimentation indicated by horizontal arrow. Vertical arrows and letters correspond to midpoints of fractions analyzed in (b). (b) Analysis of gradient fractions for DNA size: (5%) Polyacrylamide gel. Letters refer to fractions in (a); the lanes marked R and R′ are DNA fragments of control material sedimented and fractionated parallel to the experiment in (a). The R lane contains DNA of nucleoprotein complexes cosedimenting with fraction B and the R′ lane material sedimentated in the position of fraction E. The reference in the rightmost lane is a *Hin*cII digest of ϕX174 RF DNA; DNA sizes are indicated at the right.

to a continuum of longer DNA fragments (not shown).

The presence of poly(glutamic acid) in the reaction mixture overcomes the solubility problem otherwise encountered with reassembled chromatin in the presence of H1 (or H5) at near physiological ionic strengths. Thus, it is reasonable to assume that the extensive aggregation that occurs in the absence of poly(glutamic acid) could inhibit the spacing reaction. Alternatively, poly(glutamic acid) could lower the free energy for nucleosome sliding since it interacts with histone octamers and can therefore compete with DNA for octamers (Stein et al., 1979). Although poly(glutamic acid) may facilitate nucleosome sliding, it does not induce the dissociation of octamers from DNA, as measured by competition experiments with chromatin and DNA (Stein, 1980; unpublished observations). Additionally, it is possible that solutions rich in acidic polypeptides may provide an environment similar to that in the cell nucleus. In *Xenopus* oocytes, for example, the acidic protein nucleoplasmin is the most abundant nuclear protein with an in situ concentration of about 6 mg/mL (Krohne & Franke, 1980a,b; Mills et al., 1980).

In the experiment depicted in Figure 2, we used a 10-fold molar excess of H5 because this amount produced sharper DNA bands on gels than lower amounts of H5, when digestions were performed in the presence of 2 mg of poly(glutamic acid)/mL. This concentration of poly(glutamic acid) completely solubilized reconstituted chromatin under a variety of conditions. It should not be surprising that excess H5 is beneficial in the presence of large amounts of poly(glutamic acid). H1 readily exchanges in chromatin at an ionic strength of 0.1 M at 37 °C (Caron & Thomas, 1981), and poly(glutamic acid) competes with nucleosomes for H5 (the basis of our H5-stripping procedure). A 10-fold excess of H5 is not required to perform these experiments, however. For example, we used only a 1.5-fold molar excess of H1 + H5 in the experiment depicted in Figure 5, where we used a lower poly(glutamic acid) concentration.

Our data argue that H5 (or H1) has the potential to induce a new, larger spacing, apparently the native one, by moving core octamers apart in closely packed nucleosomes. This finding could be relevant to the mechanism of nucleosome spacing in vivo. Newly deposited nucleosomes in replicating chromatin appear to be closely packed for several minutes after histone deposition (Seale, 1978a,b, 1982; Levy & Jakob, 1978; Murphy et al., 1980; Jackson et al., 1981). Therefore, nucleosome "maturation", characterized by the appearance of the mature spacing, could occur as H1 (or H5) interacts with newly formed nucleosomes that contain, initially, only core histones and DNA.

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