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Acidic polypeptides can assemble both histones and chromatin *in vitro* at physiological ionic strength

(nucleosome/superhelical DNA/assembly proteins)

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ABSTRACT **We provide evidence that nucleosomes can assemble *in vitro* at physiological ionic strength (0.1–0.2 M NaCl/10 mM Tris·HCl, pH 8.0) in the absence of "assembly factors" and that poly(glutamic acid) greatly facilitates chromatin assembly under these conditions. We also show that in the presence of either poly(glutamic acid) or poly(aspartic acid), core histones assemble into octamers at physiological ionic strength. We suggest that it is a property of histones to assemble into octamers upon their interaction with macromolecules containing regions of high negative charge density, and we discuss several implications of this property.**

In eukaryotic cells, nuclear DNA is wrapped around histone octamers, forming nucleosomes (1–3). Knowledge of the mechanisms by which DNA and histones are assembled into nucleosomes will be required for an understanding of chromatin replication and, possibly, gene regulation. It is possible to reconstitute nucleosomes *in vitro* from DNA and histones by using lengthy dialysis procedures starting from 2 M NaCl in the presence or absence of urea (4–7); in contrast, direct mixing of DNA and histones at physiological ionic strength results in precipitation of the nucleoprotein, and the nature of the interactions that occur is largely obscured. Recently, Laskey *et al.* (8) were able to assemble chromatin *in vitro* at physiological ionic strength by using an extract from the eggs of *Xenopus laevis*. Furthermore, Laskey *et al.* (9) purified from this extract an acidic "assembly protein" that binds histones and transfers them to the DNA. In 0.6 M NaCl, nucleosomes form rapidly when DNA and histones are mixed in the absence of any "assembly factors"; however, a competing assembly pathway exists in which newly formed nucleosomes bind additional histones as octamers, which subsequently are transferred to protein-free DNA (10). Thus, the possibility exists that this octamer transfer mechanism observed in 0.6 M salt and the mechanism at lower ionic strength, which appears to require an assembly factor, may be related.

In this paper, we have investigated *in vitro* nucleosome assembly at "physiological" ionic strength (0.1–0.2 M NaCl/10 mM Tris·HCl, pH 8.0). We provide evidence that (*i*) nucleosomes can assemble in the absence of assembly factors, (*ii*) histones interact as octamers with acidic polypeptides, and (*iii*) poly(glutamic acid) greatly facilitates nucleosome assembly.

MATERIALS AND METHODS

Preparative Procedures. Chromatin core particles were prepared by micrococcal nuclease (Worthington) digestion of chicken erythrocyte nuclei as described (10). Salt-extracted core histones were prepared from chromatin that had been washed with 0.6 M NaCl as described (10). DNA was extracted from purified core particles with 3 M NaCl/0.05 M sodium phosphate buffer, pH 7.0, and purified with hydroxylapatite by a batch procedure. Simian virus 40 (SV40) DNA component Ir was prepared as described (11). SV40 DNA I was obtained from Bethesda Research Laboratories (Rockville, MD).

Histone Crosslinking. Samples were crosslinked in 10 mM NaCl/50 mM sodium borate, pH 9.7, 5 mg of dimethyl suberimidate per ml (Pierce), and 60 μg of histones per ml for 15 min at room temperature. Samples were electrophoresed in 5% polyacrylamide/sodium dodecyl sulfate cylindrical gels according to Weber and Osborn (12).

Agarose Gel Electrophoresis. Gels were prepared and run in the buffer system of Germond *et al.* (5). The sample buffer contained 10 mM Tris·HCl (pH 8.0), 1 mM EDTA, 10% (wt/vol) sucrose, 1% sodium dodecyl sulfate, and 0.05% bromophenol blue. The gel, 4 mm thick and 130 mm long, was run horizontally in an apparatus from Savant. Samples were electrophoresed for 8–10 hr at 50 V. Gels were stained with 2 μg of ethidium bromide per ml for 15 min and photographed under ultraviolet light (13).

Chromatin Assembly Procedure. Histones were adjusted to either 0.1 or 0.2 M NaCl by appropriate dilution of a concentrated sample in 2.0 M NaCl with 10 mM Tris·HCl (pH 8.0) 15–30 min prior to the addition of DNA. Reaction volumes were 100 μl for the reactions using SV40 DNA. A small volume of solution containing 0.7 μg of DNA was added to a dilute histone solution for all of these reactions. For the reactions requiring long incubations at 37°C, capped tubes with a total capacity of about 200 μl were used. In all cases, reaction mixtures were shaken gently. In the reactions using poly(glutamic acid) [poly(L-glutamic acid) of average molecular weight 100,000, from Miles-Yeda], histones in 0.1 M NaCl/10 mM Tris·HCl, pH 8.0, were added slowly with mixing to a 2-fold weight excess of acidic polypeptide in the same buffer. At this ratio, the mixture was initially turbid at a histone concentration of 0.6 mg/ml but cleared upon gentle shaking for several hours at room temperature. The poly(L-aspartic acid) of average molecular weight 26,000 (Sigma), used as an "assembly factor" (and in the crosslinking experiment), was less soluble. DNA was added to these preincubated histone–polypeptide complexes; 2 μl of relaxing extract, prepared from LA9 cells by the method of Germond *et al.* (5), was added last.

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Abbreviation: SV40, simian virus 40.

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RESULTS

Core Particle Reassembly. Core histones in 0.2 M NaCl/10 mM Tris·HCl, pH 8.0, were mixed in a 1:1 mass ratio with 145-base-pair DNA in the same buffer, incubated for 16 hr at 37°C, and analyzed by sucrose gradient sedimentation. Fig. 1 shows that the reconstituted nucleoprotein has a sedimentation profile very similar to that of a sample of the same material reconstituted by a standard salt-gradient dialysis procedure. In each case, approximately 55% of the total applied material absorbing at 260 nm sedimented at 11 S, the sedimentation coefficient of native core particles; 12% of the material pelleted for the sample mixed directly in 0.2 M salt, compared to 6% for the salt-gradient dialysis sample. The directly mixed sample was initially quite turbid but cleared substantially during the incubation. Samples centrifuged immediately after direct mixing gave considerably more pelleted material and less at 11 S. The protein-to-DNA ratio, measured by Lowry's method (15) with purified core particles as a standard, was 1.2 ± 0.1 (SEM) for the complex obtained by direct mixing compared to 1.1 ± 0.1 for the salt-gradient dialysis sample, as expected for reassembled core particles. Upon digestion with DNase I, both samples generated the typical pattern of DNA fragments characteristic of native core particles (not shown). These results provide both biochemical and physical evidence that complexes formed by direct mixing at physiological ionic strength are similar to those formed by salt-gradient dialysis and are, therefore, similar to native core particles (7).

Torsional Constraints. We next asked whether the direct mixing of histones and DNA at physiological ionic strength imposed the torsional constraints on relaxed, closed circular DNA that are characteristic of nucleosome formation (5). SV40 DNA (component Ir) and core histones were mixed at an ionic strength of 0.2 M, the sample was treated with topoisomerase I (relaxing extract) and deproteinized, and the DNA was analyzed for supercoiling by agarose gel electrophoresis (5). The number of superhelical turns induced is an approximate measure of the number of nucleosomes that had formed on the initially relaxed, covalently closed DNA molecules (5). Fig. 2*A* shows the distribution of superhelical species for DNA samples incubated with increasing amounts of histone. At a histone-to-DNA ratio (wt/wt) of 1.0 (lane 6), it is clear that a substantial fraction of the DNA had been completely assembled. Additionally, at lower histone-to-DNA ratios, both fully supercoiled and relaxed DNA species were observed, in contrast to what was found by salt-gradient dialysis (5) or with the *Xenopus* factor

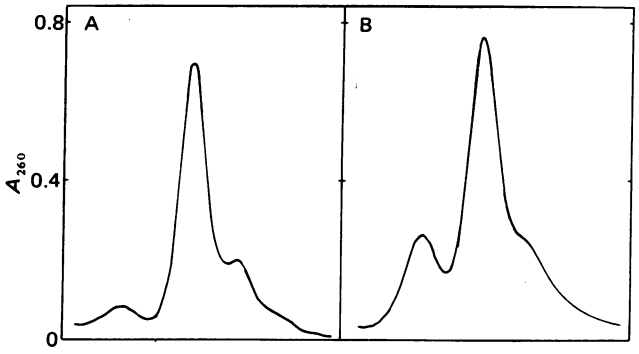

FIG. 1. Sedimentation of core particles reassembled at physiological ionic strength (*A*) or by salt gradient dialysis (*B*). DNA, extracted from purified core particles, was incubated with histones at a DNA concentration of 5 A_{260} units/ml. SW27 sucrose gradients were isokinetic for particle densities of 1.51 g/cm^3 at 4°C, C_M = 5% (14), and contained 0.2 M NaCl/10 mM Tris·HCl, pH 8.0/1 mM EDTA. Sedimentation was from left to right; peak positions correspond to 5, 11, and 15 S.

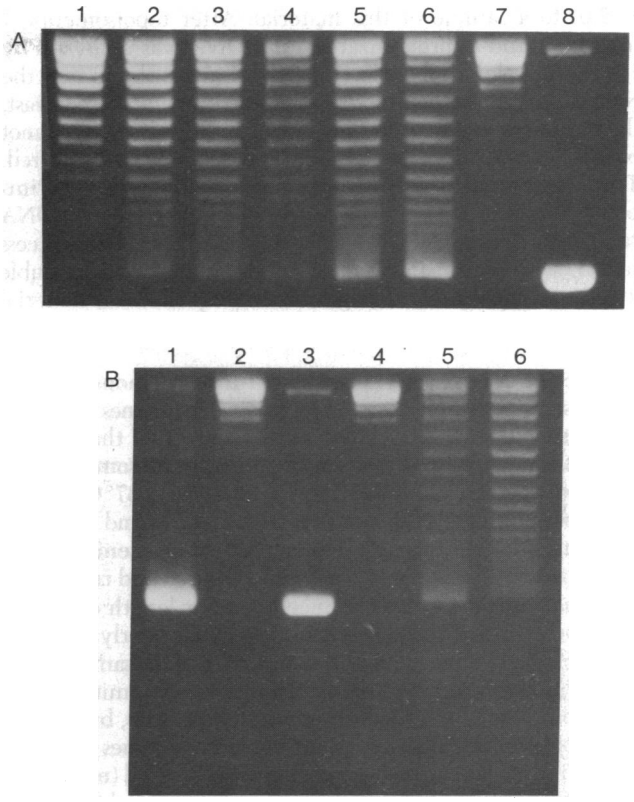

FIG. 2. Torsional constraints imposed on relaxed, covalently closed SV40 DNA upon incubation with histones. (*A*) Histones and DNA were incubated for 16 hr at 37°C in 0.2 M NaCl/10 mM Tris·HCl, pH 8.0/1 mM EDTA/0.1 mg of bovine serum albumin per ml. Relaxing extract containing topoisomerase activity was added and the samples were incubated for an additional 10 min; DNA was purified by sodium dodecyl sulfate/phenol extraction and ethanol precipitation. Histone-to-DNA ratios were: 0.2 (lane 1), 0.4 (lane 2), 0.5 (lane 3), 0.6 (lane 4), 0.7 (lane 5), 1.0 (lane 6). Lane 7 is the starting material; lane 8 is SV40 DNA I with about 22 superhelical turns. (*B*) Lane 1, SV40 component I marker; lane 2, the starting material; lane 3, minichromosomes, prepared by a salt-step procedure from 2.0 M NaCl, and treated as in *A*; lane 4, minichromosomes, as in lane 3 to which an excess equivalent of histones was added prior to treatment as in *A*; lane 5, 16-hr incubation as in *A*; lane 6, as lane 5 but the 16-hr incubation was omitted.

(8), with which all of the DNA molecules become partially supercoiled. This suggests that the assembly process at physiological ionic strength, in the absence of assembly factors, is cooperative.

In Fig. 2*B*, lane 6, relaxed DNA was incubated for a total time of only 10 min at a protein-to-DNA ratio of 1.0 in the presence of topoisomerase I, whereas in lane 5 the sample was preincubated for 16 hr before addition of topoisomerase I. Some assembly could be detected after only 10 min, but the preincubated sample was assembled to a greater extent. Thus, the 10-min incubation with relaxing extract was not responsible for all of the assembly that had occurred. This suggests that the relaxing extract is not required for nucleosome assembly, consistent with the assembly of histones and 140-base-pair DNA into core particle-like structures in the absence of relaxing extract, described above.

We observed that assembly appeared to be substantially less efficient at histone-to-DNA ratios greater than about 1.2. To investigate the effect of excess histones on nucleosome assembly, we assembled relaxed SV40 DNA into minichromosomes by a salt-step procedure from 2 M NaCl (16) and added an additional equivalent of histones (for a total histone-to-DNA ratio

of 2.0) to a sample of this material. After topoisomerase I treatment and deproteinization, the DNA was analyzed by agarose gel electrophoresis (Fig. 2*B*). Lane 3 shows that the control sample was completely assembled. In marked contrast, the sample to which excess histone was added, lane 4, cannot be distinguished from one for which no assembly had occurred. This suggests that the true degree of torsional constraint imposed cannot be accurately assessed when the histone-to-DNA ratio is greater than about 1.2. Because chromatin binds excess histone with high affinity (10, 17) and the complex is insoluble at physiological ionic strength, it is likely that this material aggregated extensively and could not be relaxed by topoisomerase I.

Electron Microscopy. In order to further demonstrate that nucleosomes assemble upon direct mixing of histones and DNA at physiological ionic strength and to show that the relaxing extract is not required to assemble minichromosomes, we incubated 1 μg of SV40 DNA (form I) for 16 hr at 37°C with 0.8 μg of core histones at an ionic strength of 0.2 M and examined the sample by electron microscopy. A field of assembled minichromosomes is shown in Fig. 3. The beaded and relaxed appearance and the greatly reduced contour length compared with protein-free SV40 DNA indicate that nearly complete assembly had occurred for at least a portion of the sample. Thus, assembly is not a consequence of proteins contaminating the topoisomerase I because none was used here. Also, by omitting the topoisomerase I treatment for minichromosomes assembled as in Fig. 2*B*, lane 3, no supercoiling was observed (not shown), indicating that the histone preparation had no nicking-closing activity. These experiments strongly suggest that only histones and DNA are required for minichromosome assembly.

Poly(glutamic acid) Greatly Facilitates Nucleosome Assembly in 0.1 M NaCl. We have observed the following: (*i*) Excess histones inhibit chromatin assembly (this paper). (*ii*) In 0.6 M NaCl, nucleosomes assemble rapidly and compete with DNA by binding additional histones; these histones are then transferred slowly to free DNA (10). (*iii*) Nucleosomes with excess histones bound are extremely insoluble in 0.1 M NaCl and induce aggregation in the sample (10, 17). Furthermore, Laskey *et al.* (9) observed that their acidic "assembly protein" was required in rather high concentrations relative to histones to facilitate assembly. These findings suggested the possibility that negatively charged macromolecules that form soluble complexes with histones might facilitate chromatin assembly in 0.1 M NaCl by providing an alternative assembly pathway that does not lead to aggregation. Thus, the transfer of histones to DNA from aggregated histone–chromatin complexes would not be expected to be very efficient, whereas the transfer of histones from soluble histone–protein complexes might be.

With this rationale, we tested whether poly(glutamic acid) facilitated nucleosome assembly in 0.1 M NaCl. Relaxed SV40 DNA was added to a mixture of histones and poly(glutamic acid); all components were at an ionic strength of 0.1 M. A 2:1 histone-to-DNA weight ratio and 2:1 poly(glutamic acid)-to-histone weight ratio were used for comparison with the assay of Laskey *et al.* (9), who reported these histone/DNA and "assembly protein"/histone ratios. Topoisomerase I was added, and the sample was incubated for 3 hr at 37°C along with appropriate control samples. The samples were then deproteinized

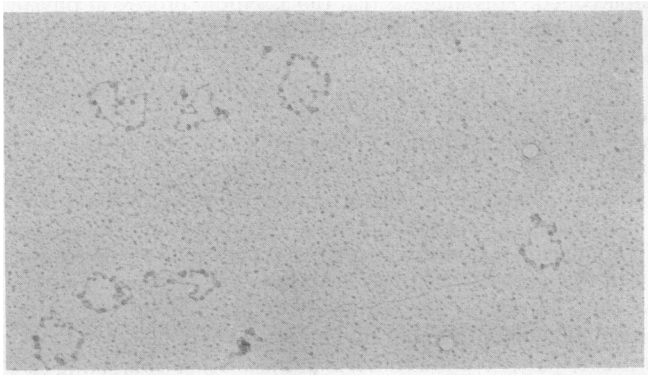

FIG. 3. Minichromosomes reassembled at physiological ionic strength. SV40 DNA I and core histones were mixed and incubated in 0.2 M NaCl/10 mM Tris·HCl, pH 8.0/1 mM EDTA/0.1 mg of bovine serum albumin per ml. No topoisomerase was added. The samples were fixed with 1% formaldehyde and adsorbed on carbon-coated grids as described (18). The samples were stained with 1% uranyl formate, rinsed with water, dried, and rotary shadowed with Pt/Pd.

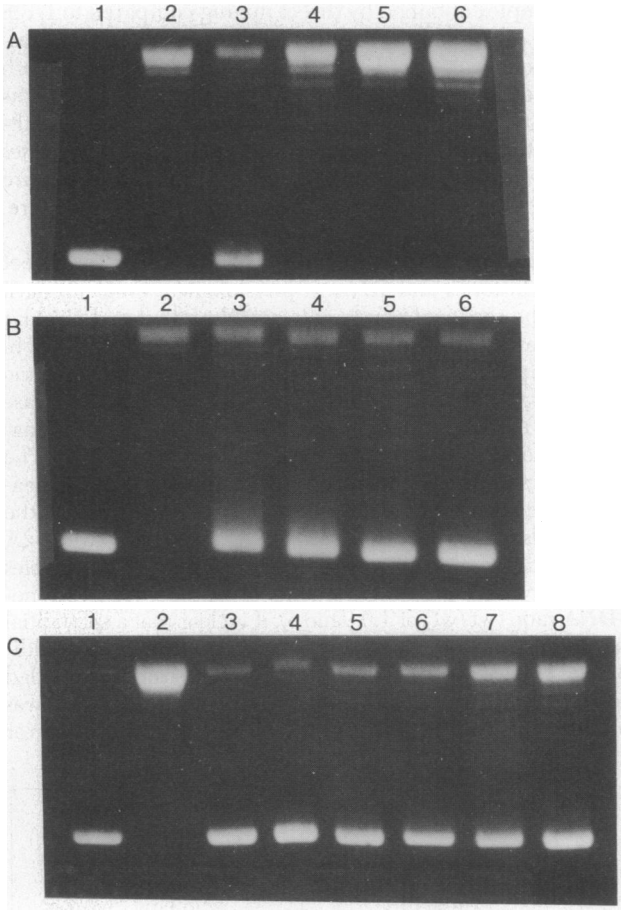

FIG. 4. Torsional constraints induced on SV40 DNA Ir upon incubation with histones and poly(glutamic acid). The incubation buffer contained 0.1 M NaCl, 10 mM Tris·HCl (pH 8.0), 1 mM EDTA, and topoisomerase. DNA samples were prepared for electrophoresis as indicated in Fig. 2. (*A*) Lane 1, SV40 DNA I marker; lane 2, starting material; lane 3, histones were premixed with poly(glutamic acid); lane 4, control, as lane 3 but poly(glutamic acid) was omitted; lane 5, control, as lane 3 but histones were omitted; lane 6, control, as lane 3 but sodium dodecyl sulfate was included. Samples were incubated for 3 hr at 37°C. (*B*) Extent of supercoiling (assembly) as a function of incubation time. Lane 1, SV40 DNA I marker; lane 2, starting material; lane 3, 5 min; lane 4, 10 min; lane 5, 20 min; lane 6, 60 min. (*C*) Optimization of the assembly reaction. The poly(glutamic acid)-to-histone ratio was kept constant at 2.0, as in *A* and *B*. Incubation time was 1 hr. Histone-to-DNA ratios were: lane 3, 1.4; lane 4, 1.6; lane 5, 1.8; lane 6, 2.0; lane 7, 2.2; lane 8, 2.4. Lane 1 contains an SV40 DNA I marker; lane 2 is the starting material.

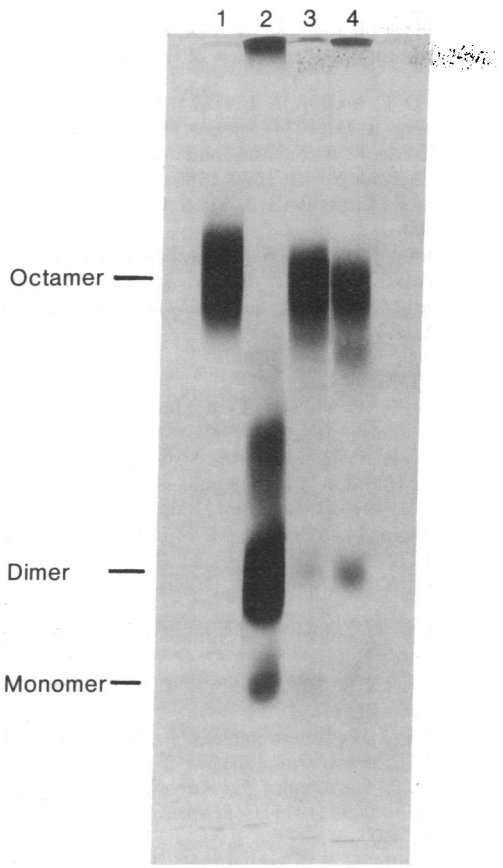

FIG. 5. Sodium dodecyl sulfate/polyacrylamide gels of histones crosslinked in the presence of acidic polypeptides at low ionic strength. Histones in 0.1 M NaCl/10 mM Tris·HCl, pH 8.0, were mixed with a 4-fold weight excess of polypeptide in the same buffer. Samples were crosslinked at a lower ionic strength. Lane 1, purified crosslinked histone octamer marker (23); lane 2, histones in the absence of polypeptides; lane 3, poly(glutamic acid) was present; lane 4, poly(aspartic acid) was present.

and the DNA was analyzed by agarose gel electrophoresis. Fig. 4*A*, lane 3, indicates that a considerable fraction of the DNA was completely assembled into minichromosomes. Lane 4 shows no apparent assembly for a sample without poly(glutamic acid) but otherwise identical [assembly in the absence of poly(glutamic acid) is inhibited due to the high histone-to-DNA ratio]. Lane 5 shows that poly(glutamic acid) in the absence of histones introduces no torsional constraints. Lane 6 demonstrates that no supercoiling occurs in the presence of the required components plus sodium dodecyl sulfate. In Fig. 4*B*, DNA and topoisomerase I were added to pre-equilibrated histone/poly(glutamic acid) mixtures and the samples were deproteinized with sodium dodecyl sulfate after an incubation for the time stated. Considerable assembly occurred in only 5 min, and the extent of assembly increased further for longer times, up to about 1 hr. This is in contrast with the results of Laskey *et al.* (8): assembly could not yet be detected after a 15-min incubation at 20°C.

We next determined the optimal conditions for chromatin assembly by varying the histone-to-DNA ratio around the arbitrary value of 2.0 while keeping the poly(glutamic acid)-to-histone ratio constant at a value of 2.0. Samples were incubated for 1 hr at 37°C. Fig. 4*C* shows that for histone-to-DNA ratios in the range of 1.4–1.6, essentially all of the DNA was fully assembled into nucleosomes, whereas higher histone-to-DNA ratios inhibited the supercoiling reaction somewhat. The bands remaining in the position of relaxed DNA in lanes 3 and 4 very likely correspond to nicked DNA, as observed by others (5, 8, 9, 18, 19). At lower histone-to-DNA ratios, assembly appears to be cooperative (not shown), as it does without poly(glutamic acid) (Fig. 2*A*).

These experiments indicate that the acidic polypeptide poly(glutamic acid) is a potent "assembly factor" for chromatin assembly at physiological ionic strength. Moreover, we found that poly(aspartic acid) also facilitated assembly (not shown).

Histones Interact as Octamers with Acidic Polypeptides. The octameric histone core of the nucleosome is unstable in solutions of physiological ionic strength; at low concentrations, histones exist predominantly as dimers (10, 20–22). Histones bind as octamers to core particles (10) as well as to DNA; both are negatively charged macromolecules. We thus suspected that histones might also bind as octamers to acidic polypeptides.

To test this hypothesis, we mixed histones with either poly(aspartic acid) or poly(glutamic acid) at an ionic strength of 0.1 M. The samples were then treated with the crosslinking reagent dimethyl suberimidate under conditions such that the ionic strength never exceeded 0.1 M. Fig. 5 shows that in the presence of either acidic polypeptide, histones were crosslinked largely into octamers, whereas under the same conditions in the absence of the acidic polypeptides histones were crosslinked predominantly into dimers; no octamer can be observed. Crosslinking between histones and the polypeptides did not occur because the synthetic polypeptides do not contain lysyl residues. In the absence of histones, bands due to poly(glutamic acid) or poly(aspartic acid) do not appear on the gel. These results suggest that histones bind to acidic polypeptides as octameric nucleosome cores.

DISCUSSION

Our results suggest that only histones and DNA are required for chromatin assembly at physiological ionic strength. It is unlikely that assembly is a consequence of nonhistone proteins in our histone preparation: (*i*) the level of nonhistone proteins in our preparation was too low to detect by sodium dodecyl sulfate/polyacrylamide gel electrophoresis, (*ii*) the histones were obtained from nonreplicating chicken erythrocytes, and (*iii*) assembly proteins do not appear to be effective in only trace amounts; for example, Laskey *et al.* (9) used 1 μg of assembly protein per 0.5 μg of histone.

The findings that histones interact as octamers with nucleosomes (10), poly(glutamic acid), and poly(aspartic acid), as well as with DNA, suggest that it is an intrinsic property of histones to assemble into octamers in the presence of macromolecules with regions of high negative-charge density. This property suggests that DNA may, on occasion, encounter preformed histone octamers. We have shown previously that DNA is capable of folding around a preformed crosslinked octamer (23), and there is evidence that chromatin core particles can partially unfold at low ionic strength by the displacement of 20–25 base pairs of DNA from each end of the particle (R. T. Simpson and H. Shindo, personal communication). Also, this property of histones is consistent with the observations that acidic proteins facilitate chromatin assembly (ref. 9 and this work). However, the major factor in facilitating assembly appears to be the increased solubility of the nucleoprotein components because DNA and crosslinked histone octamers assemble into core particles with essentially the same efficiency as DNA and uncrosslinked histones in 0.1 M NaCl in the absence of poly(glutamic acid) (unpublished observations).

This property of histones also suggests that acidic proteins could play a fundamental role in making nucleosomal DNA more accessible in chromatin. At high local concentrations of

negatively charged proteins, histone octamers could be displaced from chromatin at a low net cost in free energy because an octamer could be merely transferred to a protein that also binds it tightly. At low concentrations, negatively charged proteins would be expected to decrease the effective affinity of histones for DNA via a competition mechanism; a dynamic equilibrium whereby octamers are transiently displaced to nearby negatively charged proteins seems very plausible. Thus, negatively charged proteins may function as nucleosome "disassembly factors" as well as "assembly factors."

With these ideas in mind, it is interesting that the active regions of chromatin appear to be preferentially associated with high mobility group proteins, which contain highly acidic regions (24), and that high mobility group proteins appear to be responsible in part for the preferential DNase I sensitivity of "turned on" globin genes (25). Also, because the phosphorylation of proteins increases their negative charge, this mechanism may provide a connection between chromatin structure and the phosphorylation of nuclear porteins, which almost always accompanies increased cellular activity (26).

Our results also have implications for *in vivo* chromatin assembly. We have provided evidence that nucleosomes will spontaneously form at physiological ionic strength when nucleohistone aggregation is minimized. It is difficult to imagine how the nucleoprotein near a replication fork in the cell nucleus could aggregate in the same fashion as DNA–histone complexes in solution. Thus, although it is very plausible that an assembly factor is required *in vivo* (9), the evidence is not yet compelling that an acidic protein that simply prevents aggregation *in vitro* is advantageous in most eukaryotes. As pointed out by Laskey *et al.* (9), the *Xenopus* assembly protein could be an adaptation in this unusual system where large histone pools exist. Because histones bind strongly to chromatin *in vitro* (10, 17), it could be argued that histone-binding proteins are necessary to prevent the large excess of histones from binding to the chromatin, which may be lethal (27).

An alternative chromatin assembly mechanism has been proposed by Stein (10) whereby newly made histones interact with chromatin as excess octamers which then migrate along the chromatin to the replication fork. The observations reported here suggest that the presence of acidic proteins in the nucleus would be expected to facilitate this octamer migration because both chromatin and acidic proteins may transiently bind histone octamers. Thus, these two models for *in vivo* assembly are compatible.

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1. Olins, D. E. & Olins, A. L. (1974) *Science* **183,** 330–332.
2. Kornberg, R. D. (1974) *Science* **184,** 868–871.
3. Van Holde, K. E., Sahasrabuddhe, C. G. & Shaw, B. R. (1974) *Nucleic Acids Res.* **1,** 1579–1586.
4. Oudet, P., Gross-Bellard, M. & Chambon, P. (1975) *Cell* **4,** 281–300.
5. Germond, J. E., Hirt, B., Oudet, P., Gross-Bellard, M. & Chambon, P. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 1843–1847.
6. Camerini-Otero, R. D., Sollner-Webb, B. & Felsenfeld, G. (1976) *Cell* **8,** 333–347.
7. Tatchell, K. & Van Holde, K. E. (1977) *Biochemistry* **16,** 5295–5303.
8. Laskey, R. A., Mills, A. D. & Morris, N. (1977) *Cell* **10,** 237–243.
9. Laskey, R. A., Honda, B. M., Mills, A. D. & Finch, J. T. (1978) *Nature (London)* **275,** 416–420.
10. Stein, A. (1979) *J. Mol. Biol.* **130,** 103–134.
11. Bina-Stein, M. & Singer, M. F. (1977) *Nucleic Acids Res.* **1,** 117–127.
12. Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244,** 4406–4412.
13. Sharp, P. A., Sugden, B. & Sambrook, J. (1973) *Biochemistry* **12,** 3055–3063.
14. McCarty, K. S., Jr., Vollmer, R. T. & McCarty, K. (1974) *Anal. Biochem.* **61,** 165–183.
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
16. Oudet, P., Germond, J. E., Sures, M., Gallwitz, D., Bellard, M. & Chambon, P. (1977) *Cold Spring Harbor Symp. Quant. Biol.* **42,** 287–300.
17. Voordouw, G. & Eisenberg, H. (1978) *Nature (London)* **273,** 446–448.
18. Bina-Stein, M. & Simpson, R. T. (1977) *Cell* **11,** 609–618.
19. Keller, W. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 4876–4880.
20. Weintraub, H., Palter, K. & Van Lente, F. (1975) *Cell* **6,** 85–110.
21. Thomas, J. O. & Butler, P. J. G. (1977) *Cold Spring Harbor Symp. Quant. Biol.* **42,** 119–125.
22. Eickbusch, T. H. & Moudrianakis, E. N. (1978) *Biochemistry* **17,** 4955–4964.
23. Stein, A., Bina-Stein, M. & Simpson, R. T. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 2780–2784.
24. Levy, B., Wong, N. C. W. & Dixon, G. H. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 2810–2814.
25. Weisbrod, S. & Weintraub, H. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 630–634.
26. Kleinsmith, L. J. (1978) in *The Cell Nucleus,* ed. Busch, H. (Academic, New York), Vol. 6, Part C, pp. 221–261.
27. Nadeau, P., Oliver, D. R. & Chalkley, R. (1978) *Biochemistry* **17,** 4885–4893.