
Core nucleosomes by digestion of reconstructed histone-DNA complexes

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ABSTRACT

Reconstructed complexes of the inner histones (H2A, H2B, H3, H4) and a variety of DNAs were digested with micrococcal nuclease to yield very homogeneous populations of core nucleosomes (ν_1). Nucleosomes containing *Micrococcus luteus* DNA (72% G+C); chicken DNA (43% G+C), *Clostridium perfringens* DNA (29% G+C); or poly(dA-dT)·poly(dA-dT) have been examined by circular dichroism, thermal denaturation, electron microscopy, and DNase I digestion. Circular dichroism spectra of all particles show a typically suppressed ellipticity at 260–280 nm and a prominent α -helix signal at 222 nm. All particles show biphasic melting except ν_1 (dA-dT), which show three prominent melting transitions at ionic strength ≤ 1 mM. DNase I digestion of ν_1 (dA-dT) produces a ladder of DNA fragments differing in length by one base residue. ν_1 (dA-dT) contain 146 base pairs of DNA and exhibit an average DNA helix pitch of 10.4–10.5 bases per turn. There appear to be two regions of different DNA pitch within ν_1 (dA-dT). It is suggested that the two regions of DNA pitch might correspond to the two regions of the melting profiles.

INTRODUCTION

Many laboratories have successfully reconstructed nucleosomal structures from purified DNA and mixtures of histones (1–8). Previously we have reported physical properties of complexes reconstructed from chicken inner histones (H2A, H2B, H3, H4) and a variety of natural and synthetic DNAs (9). In this paper we describe the digestion of such complexes with micrococcal nuclease to yield very homogeneous populations of monomer nucleosomes (ν_1). We have characterized ν_1 containing several natural DNAs of different G+C content as well as the alternating copolymer poly(dA-dT)·poly(dA-dT). Particles composed poly(dA-dT)·poly(dA-dT) [ν_1 (dA-dT)] displayed a number of interesting physical properties due to their completely homogeneous base sequence.

METHODS

Preparation of Inner Histones and DNAs

Inner histones were isolated from chicken erythrocyte nuclei and stored frozen as previously described (10).

DNAs were obtained from commercial sources and used without additional purification: Micrococcus (luteus) lysodeikticus DNA (72% G+C), Sigma Chemical Corp., St. Louis, MO; chicken blood DNA (43% G+C), Calbiochem, San Diego, CA; Clostridium perfringens DNA (29% G+C), Worthington Biochemical Corp., Freehold, NJ; poly(dA-dT)·poly(dA-dT), Miles Biochemicals, Elkhart, IN; and poly(dG-dC)·poly(dG-dC), P-L Biochemicals, Inc., Milwaukee, WI.

Histone and DNA Complex Formation

DNA and inner histone were complexed by step dialysis from 2 M NaCl as previously described (9). The input of histone:DNA was 0.35 mg/ml inner histone: 0.5 mg/ml DNA (assuming 1 mg/ml inner histone has $A_{278} = 0.452$). This histone:DNA ratio allowed efficient digestion of the resulting complexes with micrococcal nuclease to monomer nucleosomes with high yields.

Digestion of Inner Histone-DNA Complexes and Purification of ν_1

Complexes were digested at $A_{260} \cong 10$ with micrococcal nuclease in 10 mM Tris-HCl (pH 7.0) and 1 mM CaCl_2 . C. perfringens, chicken, and M. luteus DNA-histone complexes were digested with 100 U/ml micrococcal nuclease for 10, 20, and 30 min, respectively. Poly(dA-dT)·poly(dA-dT) complexes were digested with 50 U/ml micrococcal nuclease for 5 min. Digestion times were decreased as the A+T% of the DNA in the complex increased, because micrococcal nuclease preferentially digests A-T regions of DNA (11). The reaction mixture was made 20 mM in EDTA and chilled on ice to stop the digestions. Digested complexes were loaded directly on 5-20% linear sucrose gradients containing 20 mM KCl and 0.2 mM EDTA (pH 7.0). Gradients were centrifuged at 41,000 rpm for 11 hr in an SW41 rotor. Gradients were fractionated on a ISCO Model 640 density gradient fractionator. Fractions containing ν_1 were concentrated, dialyzed against 0.2 mM EDTA, and used as soon as possible.

End Labeling and DNase I Digestion

To label mononucleosomal DNA at the 5'-phosphate ends, purified monomer ν_1 were incubated for 1 hr at 37° C in the following reaction mixture: 50 mM Tris-HCl

(pH 7.0), 5 mM MgCl₂, 5 mM DTT, 20 U/ml polynucleotide kinase, and 0.1 nmol [γ -³²P]ATP per 10 nmol of 5'-ends (3000 Ci/mmol, New England Nuclear, Boston, MA). The reactions were performed at a DNA concentration of 0.5 mg/ml and were terminated by making the solution 10 mM in EDTA and chilling on ice. Unreacted [γ -³²P]ATP was removed by dialysis of the reaction mixtures for 4 hr against 1 mM EDTA. The labeled v_1 were then loaded on 5–20% linear sucrose gradients containing 20 mM KCl and 0.2 mM EDTA and were centrifuged at 41,000 rpm for 10 hr in an SW41 rotor. For fractionation, gradients were dripped from the bottom of the tubes to prevent contamination with [γ -³²P]ATP at the top of the gradients. Fractions containing the labeled monomer were dialyzed against 10 mM Tris-HCl (pH 7.0), made 1 mM in MgCl₂, and digested with 25 U/ml DNAse I. The digestions were carried out at a DNA concentration of ~0.5 mg/ml. Times of digestion are given in the figure legends.

Gel Electrophoresis

DNA for electrophoresis was purified by phenol extraction and ethanol precipitation (12). DNA from monomers was electrophoresed on 5% polyacrylamide slab gels by use of the Tris-borate-EDTA buffer system (13,14). Alternatively, monomer-size DNA was electrophoresed on denaturing gels containing 7.5% polyacrylamide and 7 M urea. Gels were stained in toluidine blue and destained in water by diffusion. End-labeled DNA from DNAse I digests was electrophoresed on 40 cm, 12% polyacrylamide gels according to the procedure of Maxam and Gilbert (15). Autoradiography was done at -20°C by use of Kodak X-R-5 X-ray film.

Electrophoresis of the histones was on 15% polyacrylamide slab gels as described by Laemmli (16).

Biophysical Techniques

The methods used to study reconstructed particles (electron microscopy, circular dichroism, and thermal denaturation) have been described in previous publications from this laboratory (10). Circular dichroic and thermal denaturation data were collected and processed by a PDP-11 computer. Programs for data processing were written by M. K. Churchich.

RESULTS

Very homogeneous v_1 containing any one of a variety of DNAs were produced by

digestion of reconstructed histone-DNA complexes with micrococcal nuclease. Monomer preparations containing the following DNAs were constructed: *M. luteus* (72% G+C), chicken (43% G+C), *C. perfringens* (29% G+C), poly(dA-dT)·poly(dA-dT), and poly(dG-dC)·poly(dG-dC). Sucrose gradient profiles of digests of reconstructed complexes were very similar (Fig. 1). Digestion conditions were adjusted for each histone-DNA complex because micrococcal nuclease digests regions of high A-T much more rapidly than high G-C regions of DNA (11).

DNA purified from all v_1 , except v_1 (dA-dT), electrophoresed as a single band at ~140 base pairs on nondenaturing polyacrylamide gels (Fig. 2). DNA from v_1 (dA-dT) ran in two very sharp bands: one at ~140 base pairs and one at ~70 base pairs. We have demonstrated that this faster-moving band was due to "hairpins" formed in the self-complementary poly(dA-dT) during DNA purification. On denaturing polyacrylamide gels, the poly(dA-dT) from v_1 migrated as a single very sharp band at ~140 bases. SDS-gel electrophoresis of the various reconstructed v_1 preparations demonstrated that all inner histones were present in apparent equimolar amounts (Fig. 3).



Fig. 1. Sucrose gradient profiles of inner histone-DNA complexes digested with micrococcal nuclease. Samples were centrifuged at 41,000 rpm for 11 hr in 5-20% linear gradients. Represented are the following complexed DNAs and digestion conditions. (A) *M. luteus*, 30 min with 100 U/ml; (B) poly(dA-dT)·poly(dA-dT), 5 min with 50 U/ml; (C) poly(dG-dC)·poly(dG-dC), 60 min with 100 U/ml.

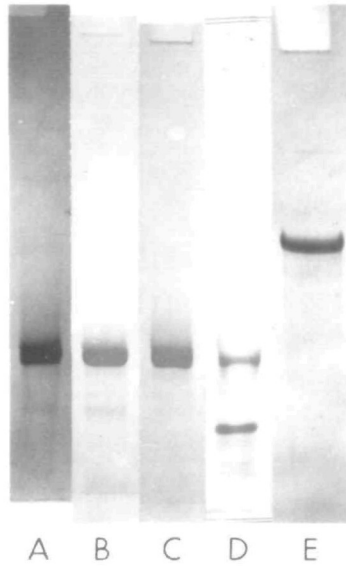


Fig. 2. 5% polyacrylamide double-stranded gels of DNA isolated from the following reconstructed v_1 . (A) v_1 (chick), (B) v_1 (*C. perfringens*), (C) v_1 (*M. luteus*), (D) v_1 (dA-dT), (E) 7.5% polyacrylamide gel containing 7 M urea of DNA from v_1 (dA-dT).

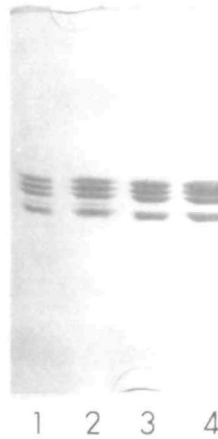


Fig. 3. SDS gel of histones isolated from v_1 (dA-dT) (lanes 1 and 2) and v_1 (chick) (lanes 3 and 4).

ν_1 (dA-dT) was observed to be the most homogeneous monomer preparation of all reconstructed ν_1 with respect to DNA length, probably because micrococcal nuclease can cut at identical sites on every particle. Micrococcal nuclease has such a strong preference for A-T regions of DNA that, in monomers of heterogeneous sequence, it may prefer to cut an A-T bond at a partially protected site rather than a G-C bond which is more exposed. Some heterogeneity of DNA length may, therefore, be inherent with nucleosomes of mixed sequence. Electron microscopy of ν_1 (dA-dT) showed them to be homogeneous and compact in appearance (Fig. 4).

We have been unable to reconstruct good monomers from poly(dG-dC)·poly(dG-dC). Soluble complexes of inner histones and poly(dG-dC)·poly(dG-dC) can be formed, and monomer particles can be clipped out at a very slow rate with micrococcal nuclease (Fig. 1C). All preparations, however, had an extensive amount of intraparticle cutting not present in the native DNA. It is possible that poly(dG-dC)·poly(dG-dC) may exist in a conformation that cannot be folded neatly around the histone core. No addi-biophysical data for ν_1 (dG-dC) will be presented in this study.

Circular dichroism

The circular dichroic spectra of all reconstructed ν_1 showed a suppressed DNA signal at 260–300 nm, minimal changes in the DNA signal at 240–260 nm, and a large α -helix contribution at 210–230 nm (Fig. 5A). The spectra of the DNAs with various base compositions were all different (Fig. 5B), and these differences were clearly reflected in the spectra of the ν_1 containing that DNA. Suppression of the circular dichroic signal from 260–300 nm, however, was a general feature of all particles. Calculation of difference circular dichroic spectra (i.e., the spectrum of a ν_1 minus the spectrum of the homologous DNA) made it possible to readily observe the general spectral features of all ν_1 (Fig. 5C).

Thermal denaturation

The derivative thermal denaturation profiles of all ν_1 reconstructed from the natural DNAs exhibited two melting transitions. Between 30 and 40% of the nucleosomal DNA melted in the first transition, and the remaining DNA melted at a higher temperature (Fig. 6A). Such biphasic melting has been previously documented in a thorough study of clean monomers (17).

The melting of ν_1 (dA-dT) was observed to be more complicated than the melting of other monomers. There were three prominent transitions when these particles were

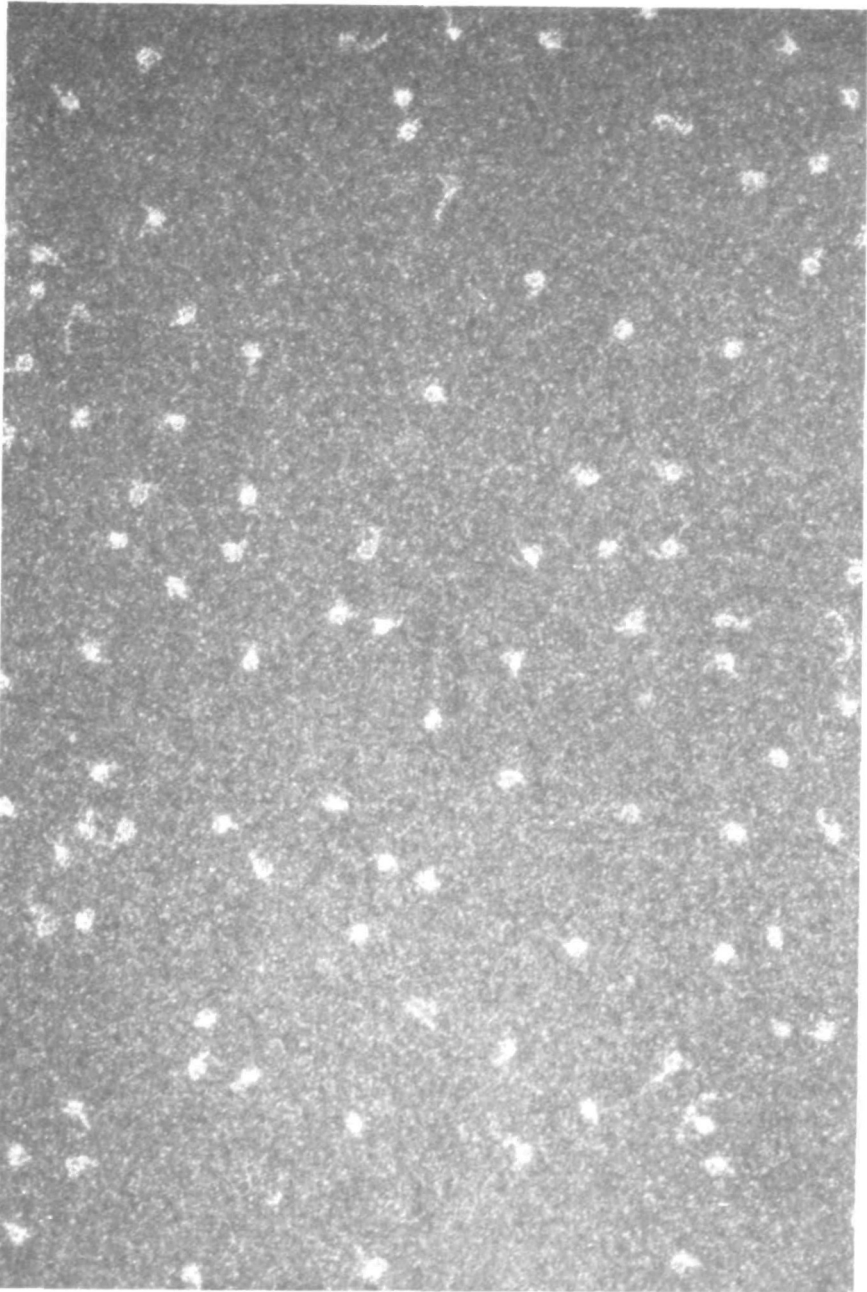


Fig. 4. Dark-field electron micrographs of v_1 (dA-dT). Samples loaded on charged carbon-coated grids without fixation, dried out of Kodak Photo-flo, and stained 30 sec with 0.1% uranyl acetate.

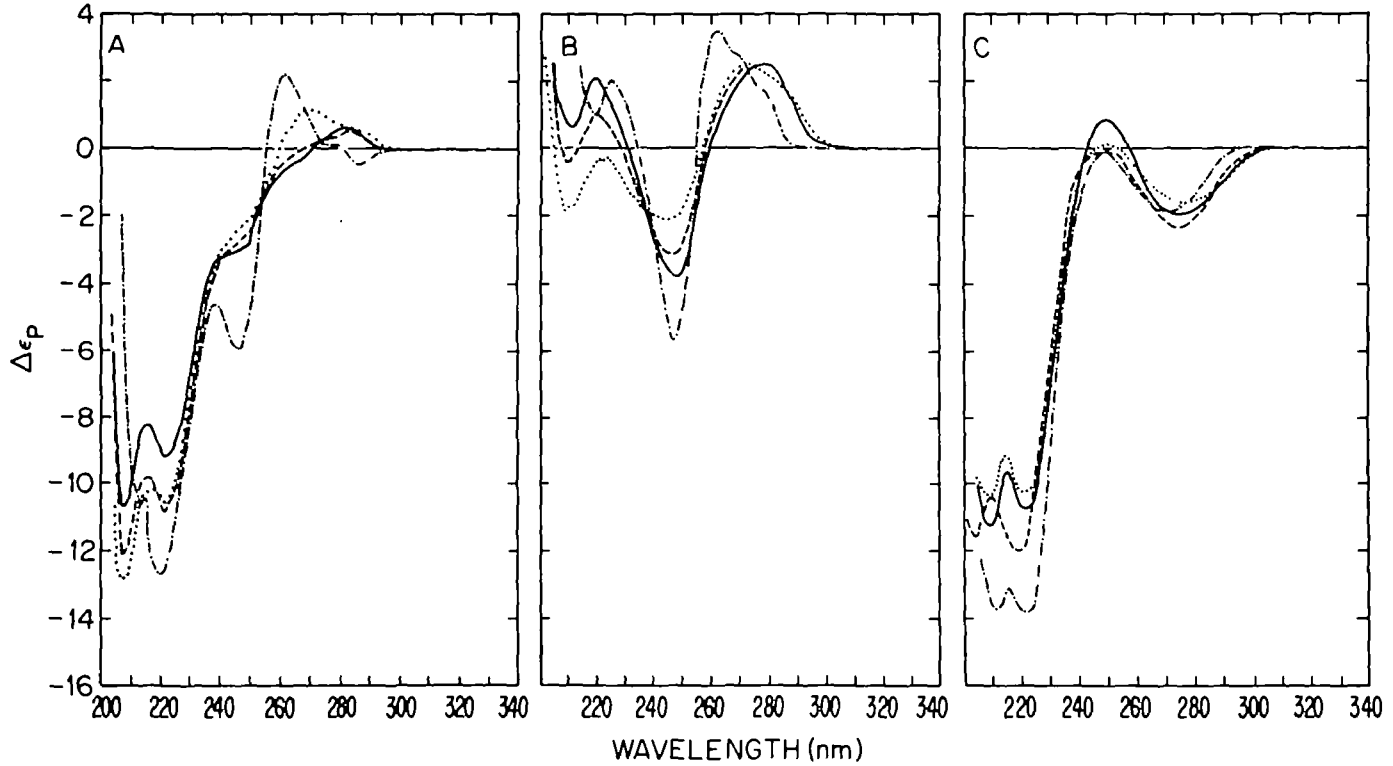


Fig. 5. Circular dichroism of (A) v_1 (chick), ---; v_1 (*C. perfringens*), —; v_1 (*M. luteus*), ···; v_1 (dA-dT), - · - ·. (B) Chick DNA, ---; *C. perfringens* DNA, —; *M. luteus* DNA, ···; poly(dA-dT)·poly(dA-dT), - · - ·. (C) Difference spectra of v_1 minus homologous DNA: chick, ---; *C. perfringens*, —; *M. luteus*, ···; poly(dA-dT)·poly(dA-dT), - · - ·. The solvent for all samples was 0.2 mM EDTA (pH 7.0) except for poly(dA-dT)·poly(dA-dT), which was in 10 mM Tris (pH 7.0).

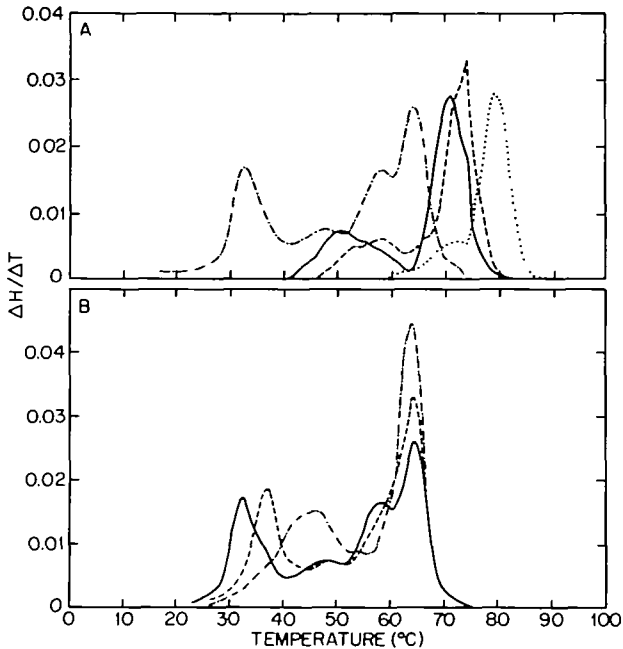


Fig. 6. (A) Derivative thermal denaturation profiles of v_1 containing the following DNAs: chicken, ---; *C. perfringens*, —; *M. luteus*, ···; poly(dA-dT)·poly(dA-dT), - · - ·. Solvent was 0.2 mM EDTA (pH 7.0). (B) v_1 (dA-dT) as a function of ionic strength (shown in parentheses): 0.2 mM EDTA (1 mM), —; 0.2 mM EDTA + 1 mM NaCl (2 mM), ---; 0.2 mM EDTA + 4 mM NaCl (5 mM), - · - ·.

melted at ionic strengths ≤ 1 mM: 30% of the DNA melted at 34°C, and ~60% melted in a doublet with peaks at 59 and 66°C. The melting temperatures (T_m) of v_1 (dA-dT) changed as a function of ionic strength (Fig. 6B). As the ionic strength was increased from 1 to 5 mM, the T_m of the low melting transition was shifted up linearly as a function of the logarithm of ionic strength. The T_m of the lower melting peak of the doublet was shifted up and fused with the highest melting peak. The position of the highest melting peak did not change between 1 and 5 mM. The change in melting profile for v_1 (dA-dT) at 1 mM ionic strength from a biphasic to triphasic form may be due to particle unfolding. Preliminary evidence from circular dichroism, sedimentation velocity measurements, and electron microscopy indicated that the v_1 (dA-dT) began to unfold extensively around 1 mM.

A comparison of the T_m of the two transitions of v_1 as a function of base compo-

sition revealed a linear dependence on G+C (%) (Table I) (18). The T_m of the highest melting transition is less sensitive to base composition [$\Delta T_m / \Delta[G+C (\%)] = 0.18$] than the low melting transition [$\Delta T_m / \Delta[G+C (\%)] = 0.5$]. The low melting transition has a G+C % dependence close to that of free DNA [$\Delta T_m / \Delta[G+C (\%)] = 0.41$] but far from that for double-stranded RNA [$\Delta T_m / \Delta[G+C (\%)] = 0.78$]. For DNA free in solution, the slope of T_m vs G+C (%) is independent of ionic strength (19). This suggests the possibility that the two melting transitions of ν_1 are not simply in different ionic environments. By analogy with the different G+C (%) dependence of the T_m of different helical forms of nucleic acids, the DNA in ν_1 may be in two different conformations.

DNase I digestion

When monomers containing natural DNAs were digested with DNase I, a ladder of single-stranded fragments was produced with fragment lengths occurring in multiples of ten bases. This digestion pattern has been documented in many laboratories to be characteristic of nucleosomal structure (7,20,21). The pattern probably arises because of the periodicity of the DNA helix. The pitch of B-family helices is about 10 bases/turn, thus a favored site for DNase I cutting becomes available with every turn of the helix.

Digestion of ν_1 (dA-dT) with DNase I produced a ladder of single-stranded fragments which were multiples of two bases in length (Fig. 7). Every fifth or sixth band on the gel was strong in intensity, indicating that the preferred sites of cutting were somewhat more than 10 bases apart. The intensity of the bands fell off symmetrically about the preferred sites of cutting. Cuts occur at multiples of two bases, because DNase I

TABLE I. Melting temperatures for various DNAs and ν_1

Type of DNA	G+C (%)	Melting temperature (°C) for		
		DNA	ν_1 , low transition	ν_1 , high transition
Poly(dA-dT)· poly(dA-dT)	0	17.2	34	66
<u>C. perfringens</u>	29	43.6	51	71
Chicken	43	49.3	58	73
<u>M. luteus</u>	72	61.2	72	79

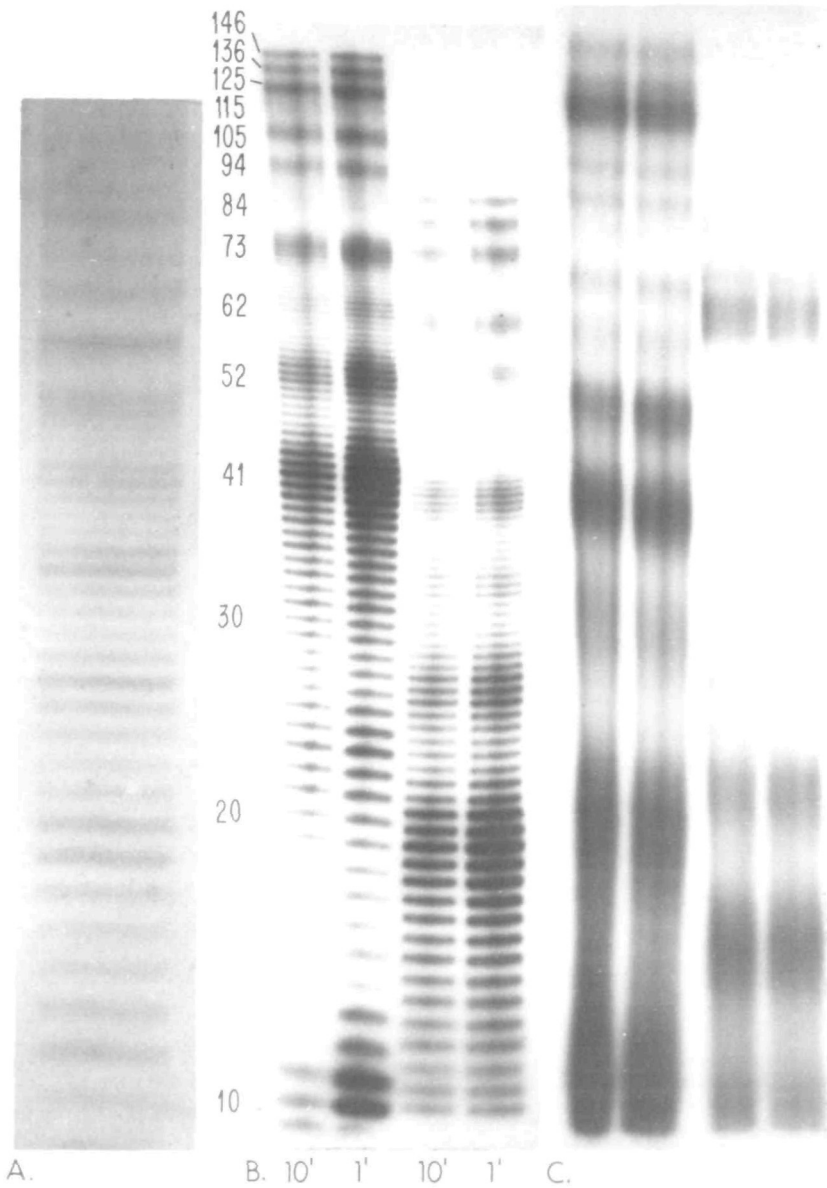


Fig. 7. (A) DNA from $v_1(dA-dT)$ digested with 25 U/ml DNase I for 10 min, run on 7.5% polyacrylamide gels containing 7 M urea, and stained with toluidine blue. (B) $v_1(dA-dT)$; 1- and 10-min digests with 25 U/ml DNase I. (C) Native chicken v_1 ; 1- and 10-min digests with 25 U/ml DNase I.

preferentially cuts the A-pT bond rather than the T-pA bond (22). Any two cuts within a particle will produce a fragment with an even number of bases. Fragments resulting from multiple internal cuts will therefore be multiples of two bases in length.

When ν_1 (dA-dT) were 5'-end-labeled with ^{32}P and digested with DNase I, autoradiographs of denaturing gels showed a one-base ladder of DNA fragments (Fig. 7B). All fragments are generated in end-labeled particles because either A or T can fall at the end of a DNA strand and become labeled. The strands in which A is labeled yield fragments with an odd number of base residues, and strands in which T is labeled give rise to fragments with an even number of bases. If a series of gels is run to resolve different regions of the digestion pattern, it is possible to count every band in the particle. Counting bands gives an accurate value for the DNA length in the ν_1 (dA-dT) particle — 146 base pairs.

The number of bands between peaks of cutting in the particle may be interpreted as revealing the pitch of the DNA helix as it winds around the histone core. The average pitch of the helix was measured at ~10.5 bases/turn. There were, however, differences in the helix pitch in different regions of the particle: in the central region (41–105 bases) the calculated pitch was 10.67; at the ends (0–41 bases and 105–146 bases) the pitch was 10.25.

A comparison of the frequency of cuts from the 5'-ends of ν_1 (dA-dT) with a digest of native chicken ν_1 (Fig. 7C) showed the patterns to be very similar. Strong cutting sites occurred at 41 and 52 bases, and weak sites occurred at 30, 84, and 115 bases. Differences in the relative frequency of cuts occurred only at the 5'-end (0 – ~22 bases). These sites were cut less frequently in ν_1 (dA-dT) than in native ν_1 . This is likely due to strands at the ends of ν_1 (dA-dT) melting away after being nicked and then being further cut nonspecifically to small oligomers. When ν_1 (dA-dT) are digested under conditions in which no melting would occur (25°C, 1mM MgCl_2 , 10 mM Tris-HCl) the cutting pattern at the 5'-end returns to normal.

The DNA fragments from ν_1 (dA-dT) were well resolved in gels, because fragments in any given band were of identical sequence. In mixed sequence nucleosomes, fragments differ in sequence, which affects their migration in the gel and causes resolution of bands to be poor. Even in native ν_1 , however, individual bands can be seen between 10 and 20 bases, where resolution was optimal.

DISCUSSION

Monomers prepared by digestion of histone-DNA complexes were homogeneous with respect to DNA length and exhibited clear nucleosomal structure as determined by circular dichroism, thermal denaturation, DNase I digestion, and electron microscopy. It was possible to obtain good reconstruction between chicken erythrocyte inner histones and DNA of a wide variety of G+C %. In principle one can incorporate almost any type of native or modified DNA (e.g., bromo-substituted, methylated, UV-irradiated, or labeled) into the nucleosome. Some DNAs [e.g., poly(dG-dC)·poly(dG-dC)] do not appear to form good nucleosomal structure, however. In addition, we have been unable to form nucleosomes by combining inner histones with the double helical RNA poly rI·poly rC which is in the A conformational form.

Circular dichroic properties of nucleosomes studied as a function of base composition exhibited suppression of the spectra from 260-300 nm. This suppression was a general feature of nucleosome structure, largely independent of the specific DNA conformation due to base composition. It is not known whether the common changes in circular dichroic properties which occur when DNA is folded into ν_1 result from changes in DNA secondary structure or from tertiary interactions between adjacent loops of DNA (23). The circular dichroic contributions at 222 nm showed that all ν_1 had comparable amounts of α -helix.

Thermal denaturation studies showed that the two melting transitions of nucleosomes had different dependence on G+C (%). The lower melting transition was more sensitive to G+C (%) (and more similar to free DNA in this respect) than the higher melting transition. This gave some indication that the DNA in the nucleosome may be in two different conformational states.

ν_1 (dA-dT) showed a typical, biphasic melting profile at ionic strengths above 2 mM. At 1 mM ionic strength the higher melting transition split into a doublet. The change in melting may reflect the structural transition seen between 1 and 2 mM in the sedimentation and diffusion of native ν_1 (24). Possibly melting differences in this range of ionic strength are seen only in ν_1 (dA-dT) because of higher resolution in the melting profile afforded by these particles. Preliminary evidence from electron microscopy, circular dichroism, and sedimentation indicated, however, that ν_1 (dA-dT) was less stable to low ionic strength than ν_1 containing natural DNAs. The change in melting may therefore reflect a more extensive unfolding of ν_1 (dA-dT).

DNase I digestion of 5'-phosphate end-labeled ν_1 (dA-dT) produced a one-base ladder of digestion products when electrophoresed on denaturing gels. Every DNA band in the particle could be resolved. The DNA length of ν_1 (dA-dT) was accurately determined at 146 base pairs. The average helix pitch of DNA on the particles was ~ 10.5 bases/turn. There were, however, two regions apparently of different pitch within the particles: in the central regions (41–105 bases) the pitch was 10.67; at the ends the pitch was shorter — 10.25 bases/turn.

The pattern of peaks of cutting suggested a symmetry about the 73 base site. There were 32 bases between peaks of cutting at 41 and 73 and between 73 and 105 base sites. Thus DNA with a pitch of 10.67 was apparently distributed symmetrically about the 73 base site. There were no clear peaks of cutting at either 31 bases or 115 bases because these sites were so protected. As a consequence of this, we could not tell exactly where the longer pitch of the central region ended and the shorter pitch of the ends began. With the 41 and 105 base sites as points of reference, there were 41 bases on both ends of the particle, giving a pitch of 10.25 bases/turn. The different helix pitches are thus distributed symmetrically about the 73 base site.

Calculation of pitch from the number of bands at the ends of the particles must take into consideration whether the DNase I and micrococcal nuclease sites coincide. According to Sollner-Webb *et al.*, the two enzymes cut at two different sites which are symmetrically located about a common dyad (25). They predict that there should be 12 bases between the 5'-end of a particle and the first visible DNase I cut. On the 3'-end they predict there should be eight bases between the first DNase I cut and the end of the DNA strand. In ν_1 (dA-dT), however, there appeared to be 10 bands between the first DNase I cut and the end of the particle DNA on both the 3'- and 5'-ends. Thus, the micrococcal nuclease and DNase I cutting sites appeared to coincide. We are currently investigating this point further. Even if the 0–10 and 136–146 base regions of the ladder were ignored, however, the helix pitch at the ends of the particles was significantly less than the pitch in the central region.

When ν_1 (dA-dT) were digested at 37°C, the cutting frequencies on the 5'-ends of the particles (0–22 bases) were much less prevalent than expected by comparison with the cutting frequencies of native chicken ν_1 . The gel pattern at the 3'-ends, however, was normal with respect to both cutting frequencies and the pattern of peak intensities.

These data can be interpreted in terms of the instability of the dA-dT helix.

The 37°C temperature used for DNase I digestion is close to the T_m of the lowest melting transition of ν_1 (dA-dT) under the ionic strength conditions used for digestion. Studies of native ν_1 indicate that this low melting transition may be caused by the melting of the DNA at the ends of the nucleosomes (17). A nick near the ends of the ν_1 (dA-dT) may, therefore, cause the end of that strand to melt away and become susceptible to nonspecific cleavage to small oligomers. Some cuts at sites between the 5'-end and ~22 bases would thus be lost to detection. The strong protein-DNA interaction centered around the 30 base site could prevent the loss of fragments longer than ~22 bases. Above 22 bases, therefore, the gel pattern would be normal. The loss of part of a strand due to nicks on the 3'-ends would have no effect on the gel pattern, since only fragments containing the 5'-end label were detected.

This explanation predicts that the relative intensity of bands below 23 bases would be even lighter at long digestion times. This is what is seen when a 1-min digest is compared with a 10-min digest (Fig. 7B). Normally one would expect an accumulation of smaller fragments at longer digestion times. This explanation is further reinforced by the fact that when digestion is carried out at 25°C (well below the early melting transition under these ionic conditions) the cutting pattern is restored to normal both with respect to cutting frequency and pattern of peak intensity.

The melting and DNase I digestion data can be integrated into the model of Finch *et al.* (26) (Fig. 8). Although one cannot tell exactly where the longer pitch of the central region of ν_1 (dA-dT) ends and the shorter pitch begins, the data are consistent with the following explanation. The central region of the DNA is clamped by strong protein interactions at the 30 and 115 base sites into a superhelical turn containing ~85 base pairs of DNA. The pitch of the DNA in this region is 10.6-10.7 bases/turn. The DNA at the ends of the particles is less constrained and assumes a pitch of ~10.2 bases/turn. These 60 bases at the ends of the particles could be responsible for the low melting transition, and the main melting transition is probably due to the DNA in the central region of the nucleosome. The reason for the two apparent DNA conformational domains in ν_1 remains uncertain. The presence of the free DNA ends generated by micrococcal nuclease may release some of the strain of supercoiling and cause a change in the DNA conformation at the ends of ν_1 . Alternatively, the DNA at the ends of the particles may be less tightly bound to histone than the DNA in the central regions (27). This may allow the DNA more freedom to writhe and thus cause a change in helix pitch. It should be noted that the melting of the nucleoprotein regions in complexes of inner

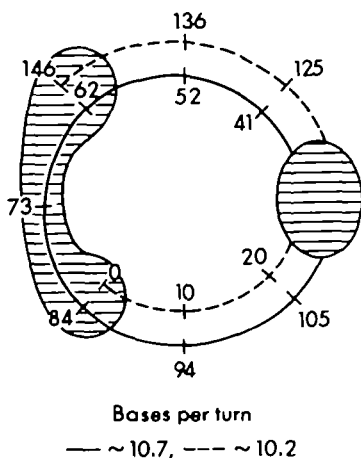


Fig. 8. Representation of superhelical, nucleosomal DNA with 85 base pairs per turn. Numbering is from the 5'-end of one strand. Shaded areas represents regions of low to medium cutting. Adapted from Finch *et al.* (26).

histone and long DNAs is biphasic (9). Furthermore, the melting temperatures of both transitions are independent of the histone:DNA ratio. This reinforces the idea that different regions of histone-DNA interactions, rather than free DNA ends, are responsible for the two DNA conformations in ν_1 .

Possibly conformational changes due to biological processes are greater at the ends of nucleosomes. We are currently investigating the effect of biological perturbants of structure (i.e., acetylated histones and nonhistone proteins) on the conformation of the DNA in ν_1 (dA-dT).

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