Proc. Natl. Acad. Sci. USA
Vol. 75, No. 8, pp. 3583–3587, August 1978
Biochemistry

Compact oligomers and nucleosome phasing

(chromatin/nuclease digestions)

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Communicated by Gary Felsenfeld, April 28, 1978

ABSTRACT **Micrococcal nuclease (EC 3.1.4.7) digestion of histone H1- and H5-depleted chicken erythrocyte chromatin yields, in addition to 140-base-pair (bp) core particles, a series of nucleosome oligomers containing about 260 bp (compact dimer), 380 bp (compact trimer), etc. of DNA. These are postulated to represent members of a class of oligomers in which the DNA is tightly wound on stacked protein cores. The physical properties (melting, circular dichroism) as well as DNase I (EC 3.1.4.5) digestion patterns support this view. DNase I digestion of tight oligomers in which the 5′ ends of the DNA have been labeled yields results consistent with this model and inconsistent with some other possible models. Several classes of such particles are postulated to exist, differing in DNA length by 10-bp increments. This may be an explanation of the 10-bp nucleosome "phasing" that has been observed in some nuclei.**

The basic repeat unit of chromatin, the nucleosome, is thought to consist of the core particle, which contains 140–160 base pairs of DNA (bp) in intimate contact with core histones, plus a spacer or linker region of variable DNA length. In a recent paper (1), we noted the existence of "compact" oligomers of the nucleosome in micrococcal nuclease digests of chicken erythrocyte chromatin that had been depleted of histones H1 and H5. Recently, Klevan and Crothers (2) have reported the isolation and characterization of a nucleosome dimer containing only 240 bp of DNA. In this paper we describe the isolation and detailed characterization of a class of such nucleosomal oligomers (compact oligomers) from H1- and H5-depleted erythrocyte chromatin and give a better definition of their DNA sizes and structures.

DNase I (deoxyribonucleate 5′-oligonucleotidohydrolase, EC 3.1.4.5) digestion of these oligomers yields a 10-base (b) ladder of DNA fragments (1, 3) extending to 270 b in the dimer and at least 340 b in the trimer. Judging from their physical structure and composition, these oligomers have no DNA spacers; in fact, they represent structures in which two protein cores share interactions with some regions of DNA.

Although we have no evidence for the existence of such particles *in vivo*, they represent a most interesting type of possible chromatin structure.

MATERIALS AND METHODS

Preparation and Digestion of Chicken Erythrocyte Chromatin. Blood was obtained from male White Leghorn chickens by exsanguination. Erythrocytes and nuclei were prepared as described by Shaw *et al.* (4) with modifications given by Tatchell and Van Holde (5). Chromatin was depleted of H1 and H5, digested with micrococcal nuclease (nucleate 3′-oligonucleotidohydrolase, EC 3.1.4.7) (Worthington) and run on sucrose gradients as described (5). Those fractions running ahead of the large core-particle peak were rerun on sucrose gradients. Fractions corresponding to compact oligomer particles were dialysed against 10 mM Tris-cacodylate/0.7 mM EDTA, pH 7.2. All experiments were carried out in this buffer unless stated otherwise.

Physical Characterization. Thermal denaturation in 1 mM cacodylic acid (pH 7.2) was carried out as described (6). The circular dichroism (CD) spectra were recorded with a Jasco JSP CD apparatus calibrated against camphor sulfonic acid. Analytical velocity sedimentation was done in a Beckman model E ultracentrifuge equipped with scanner optics as described (5). Protein concentrations were determined by the method of Lowry with modifications by Hartree (7); DNA concentrations were measured by the absorbance at 260 nm.

End Labeling and DNase I Digestion. T4 kinase was prepared from T4 lysate according to Richardson (8) and purified by phosphocellulose chromatography. The particles were labeled with ^{32}P as described by Simpson and Whitlock (9) to a specific activity of 10^6 cpm/μg of DNA by using [γ-^{32}P]ATP. The particles were diluted with nonlabeled particles to 5×10^3 cpm/μg and then digested with DNase I (100 units/ml) at 37° in the presence of 0.7 mM Mg^{2+} at a concentration of 50 μg of DNA per ml. The reaction was terminated by addition of Na_2EDTA to 10 mM, and the DNA was extracted by using the modified Marmur procedure, precipitated with 2.5 vol of ethanol, and redissolved in electrophoresis buffer.

Electrophoresis. ^{32}P-End-labeled DNA fragments from the DNase I digestion were run on 2 mm × 25 cm 8% polyacrylamide denaturing slab gels according to Maniatis *et al.* (10) at 300 V for 6–16 hr. Each well was loaded with 25–30 μg of DNA carrying 10^5 cpm of ^{32}P so that each well could be both stained with ethidium and autoradiographed. The gels were stained with ethidium bromide and photographed as described by Shaw *et al.* (4). The gels were then autoradiographed by using Kodak X-omat R film. The Polaroid type 55 negatives and autoradiograms were scanned with an Ortec densitometer.

Gels for double-stranded DNA were run according to Loening (11) and gels for protein were run as described by Laemmli (12) or Alfageme *et al.* (13), with the apparatus designed by Matsudaira and Burgess (14) in the former case.

RESULTS

The compact oligomers were isolated from a micrococcal nuclease digestion of H1-depleted chromatin as described by Tatchell and Van Holde (5). After digestion to 20–30% acid-soluble DNA, more than 90% of the remaining chromatin existed as 140-bp core particles. The remaining small fraction of soluble chromatin existed as a set of stable particles containing DNA in two main size classes: a 260- to 270-bp class, and a 380- to 400-bp class. We call these particles "compact dimer" and "compact trimer," respectively. In some digests we have seen a DNA band at about 520 bp that would correspond to a compact tetramer. We have obtained small quantities of this oligomer, allowing partial characterization. It should be empha-

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Abbreviations: bp, base pairs of DNA; b, bases of single-stranded DNA; CD, circular dichroism.

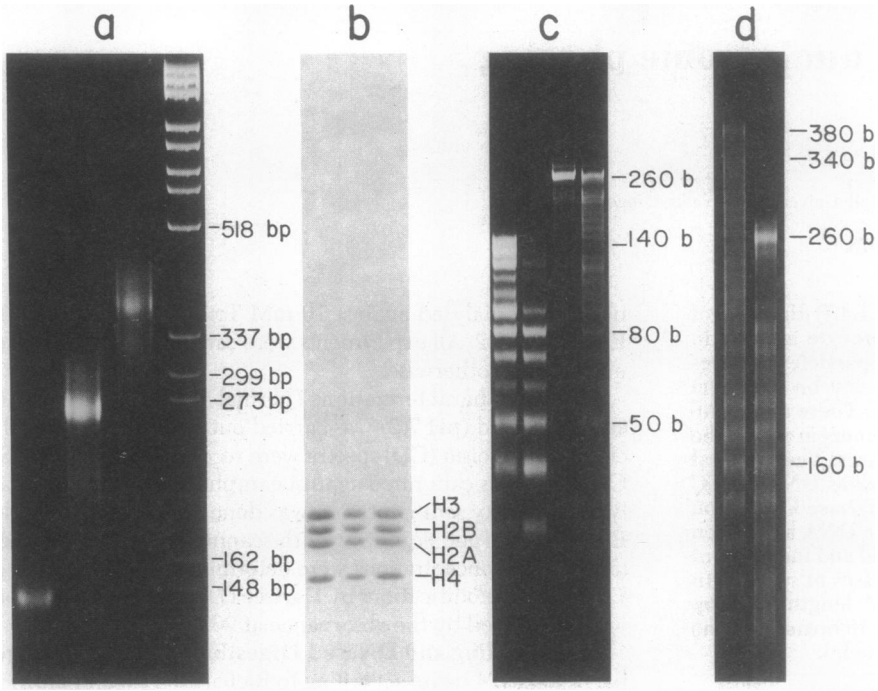

FIG. 1. (*a*) A 4.5% polyacrylamide nondenaturing gel showing DNA isolated from (left to right): core particles, compact dimers, compact trimers, and PM2 *Hae* III restriction fragments. Electrophoresis was from top to bottom. (*b*) Na-DodSO$_4$/15% polyacrylamide gel showing proteins from (left to right): core particles, compact dimers, and compact trimers. (*c*) Urea/8% polyacrylamide denaturing gel of DNase I digested core particles and compact dimers. From left to right: 1-min and 5-min digestions of core particles; 0 digestion and 20-sec digestion of compact dimers. (*d*) Urea/8% polyacrylamide denaturing gel of DNase I-digested compact trimer (20-sec digestion) and compact dimer (1-min digestion). The DNA smaller than 110 bp was run off the gel to allow better resolution of the higher molecular weight bands.

sized that this class of oligomers represents structures with very much smaller DNA than those normally found on brief micrococcal nuclease digestion of chicken erythrocyte nuclei. The average repeat for chicken erythrocyte chromatin is about 200 bp (4, 15).

The exact sizes of DNA in the compact dimer and trimer are important in that the length of DNA in these structures will profoundly influence the possible models that can be proposed. The sizes of *Hae* III restriction fragments of PM2 DNA, used as standards, have been redetermined, using sequenced fragments of simian virus 40 DNA as primary standards.* The data for this recalibration were kindly provided by R. T. Kovacic (personal communication). The results were in good agreement with sizes calculated by Noll (16) but were slightly larger than sizes previously reported from this laboratory (17). The availability, in the last year, of a set of sequenced DNA fragments has allowed a more accurate calibration.

On high-resolution polyacrylamide gels, the compact dimer DNA was a band centered at 265 ± 10 bp; the trimer DNA was at 390 ± 20 bp (Fig. 1*a*). The value, less accurate, for the tetramer DNA was 520 ± 40 bp. When compared with the homogeneous restriction fragments, it is obvious that, although there is some heterogeneity in the DNA lengths of the compact oligomers, they are far more homogeneous than are oligomers prepared by the usual technique of digestion of chromatin in nuclei. On denaturing gels, the compact dimer DNA was resolved into two sharp bands at 260 and 270 b. The trimer DNA seemed to be resolved into bands at 380, 390, and 400 b, although the resolution was poor. We have not so far been able to observe such resolution of the oligomer DNA into bands on nondenaturing gels. A possible explanation is that some oligomers contain duplex DNA with different length strands—i.e., a compact dimer with one 270-b DNA strand and one 260-b strand. Another possible explanation is that base sequence and composition influence the migration of double-stranded DNA on polyacrylamide gels. We have some evidence for this from the abnormal migration of certain sequenced restriction fragments on nondenaturing polyacrylamide gels.

When compact oligomer particles were isolated from preparative sucrose gradients, each sedimented with a homogeneous boundary in the analytical centrifuge. The $s_{20,w}$ values are presented in Table 1. The value for the compact tetramer may be in error due to slight proteolysis in this sample. Compact oligomers have the same histone stoichiometry as nucleosomes or core particles; roughly equal amounts of histones H3, H2B, H2A, and H4 were found but no H1 or H5 (Fig. 1*b*) and no modified histones were indicated by electrophoresis on acid/urea/Triton/polyacrylamide gels (13) (data not shown). Thus, as far as we can see there is nothing unusual about the protein composition. The protein/DNA ratios presented in Table 1 indicate that the dimer contains two octamers of histones (four each of the inner histones) whereas the trimer contains three octamers of histones.

The compact oligomers showed a higher melting temperature than the core particles when thermally denatured. A first-derivative plot of the denaturation is shown in Fig. 2. Although the melting profiles are complex it is interesting that the values at the major transition of the compact oligomers are higher than for the cores, and the fraction of DNA associated with the lower melting transition is decreased.

The CD spectra of core particles, compact dimers, and compact trimers are presented in Fig. 3. The compact oligomers had a greater negative ellipticity at 295 nm, diminished positive ellipticity at 280 nm, and increased positive ellipticity at about 270 nm, as compared to the core particles. A preliminary experiment with compact tetramers indicated (not shown) that this trend continues, the increase at 270 nm being most marked.

Digestion of compact oligomers by pancreatic nuclease gave the extended ladder of DNA fragments as shown in Fig. 1 *c* and *d* and Fig. 4. With high-resolution gels, the bands could easily be resolved to up to 270 b in the dimer and to 340 b in the trimer; in all likelihood, they continued to 380 or 390 b in the latter case (Fig. 1*d*). Just as with the digestion of nuclei *in situ* with pancreatic nuclease, the DNA bands in the compact oligomers were of unequal intensity, with the 80-b band being most intense. Higher molecular weight bands also showed a reproducible variation in intensity.

* Sizes of *Hae* III restriction fragments of bacteriophage PM2 DNA: H, 518 bp; I, 337 bp; J, 299 bp; K, 273 bp; L, 162 bp; M, 148 bp; N, 120 bp; and O, 97 bp.

Table 1. Properties of compact oligomers

	DNA size, bp*	Protein/DNA†	$s_{20,w}$ Obs.‡	$s_{20,w}$ Pred.§	% premelt	bp DNA in premelt
Core particle	144 ± 5	1:1 ± 0.1	10.9 ± 0.1	(10.9)	29	40
Compact dimer	265 ± 10	1.2 ± 0.1	15.4 ± 0.3	15.8	20	53
Compact trimer	390 ± 20	1.2	19.4 ± 0.4	19.1	14	55
Compact tetramer	520 ± 40		22.8	22.9		

* ± approximately half-width of main peak.
† Shown as wt/wt ratios; means of multiple samples from two preparations (±SD) for core and dimer and one for trimer.
‡ Observed average of three experiments (±SD) for core, dimer, and trimer; one measurement for tetramer. All values in svedbergs.
§ Predicted by assuming $s_{20,w}$ for core to be 10.9 S. See text.

We labeled the 5′ ends of the DNA in the compact oligomers with ^{32}P and followed these ends in the pancreatic nuclease digestion by autoradiography of the DNA gels in a manner similar to that used by Simpson and Whitlock (9). Scans of the autoradiographs are represented by dotted lines in Fig. 5. The distribution of ^{32}P in DNA bands below 140 b (not shown) supports Simpson and Whitlock's results and is nearly the same in core, compact dimer, and compact trimer digests. The pattern of labelled bands above 140 b in the dimer and trimer will be discussed below.

DISCUSSION

At first it seemed remarkable to us that particles such as these compact oligomers could exist at all. If the 140-bp core particle is in fact the basic unit of chromatin structure, the smallest dimer and trimer might be expected to contain 280 and 420 bp of DNA, respectively. However, after carefully considering the model proposed by Finch *et al.* (18) for the core particle, we believe that there is a simple explanation that is consistent with all of the available data.

In the model of Finch *et al.*, the 140-bp DNA makes 1.75 turns about a 70-Å-diameter protein octamer. The DNA is assumed to complete one turn per 80 bp, with a pitch of 28 Å. Pardon *et al.* (19) have proposed that the histone octamer core is a squat cylinder, 70 Å in diameter and 35–40 Å high. Putting these data together, one arrives at the model of the core particle shown in Fig. 6*a*. The dimensions of this particle immediately suggest a model for our compact oligomers (20). The compact (260 bp) dimer can be visualized by stacking cores about a (pseudo) dyad axis, as shown in Fig. 6*b*. In such a particle, the stacked cores are practically in contact and are rotated 180° with respect to one another about the superhelix axis. If the DNA ends are trimmed to the same extent as in the core particle, such a particle will contain *just 260 bp of DNA*. Furthermore, the corresponding trimer will have 380 bp of DNA. In fact, these particles are members of a series, of which each member (n) contains ($120n + 20$) bp of DNA coiled in a uniform superhelix of pitch 28 Å. This model requires each histone octamer to interact with less than the usual 140 bp of DNA; that is, some DNA is shared between octamers. This assumption is reasonable in light of evidence that core particles are stable with a DNA size as small as 120 bp (21, 22) and that the strong DNA–histone interactions start 20 b in from the ends of the DNA (6, 23).

It is clear, however, that this series represents only one class (the most compact) of a number of kinds of particles in which protein cores are closely stacked. The next series can be generated by inserting DNA symmetrically about the new dyad axis. But, if the protein cores are to remain stacked in a *parallel* array, this extra DNA can be inserted *only* in 10-bp increments. This is required because the ends of the DNA strands in the inserted segment must match. Any other length (say 7 bp, for example) will lead to either a twisting of one core with respect to the next or a deformation of the DNA if the histone cores remain parallel. Thus, the next such dimer should have 270 bp, the next 280 bp, etc. We believe that this requirement could explain some or most of the observed "extended ladder," or "nucleosome phasing" found in chromatin from various sources (1, 24). The explanation is consistent with the observation that the extended ladder is best resolved with chromatins (like yeast) that display a short average repeat (1).

We think that a number of experimental results strongly

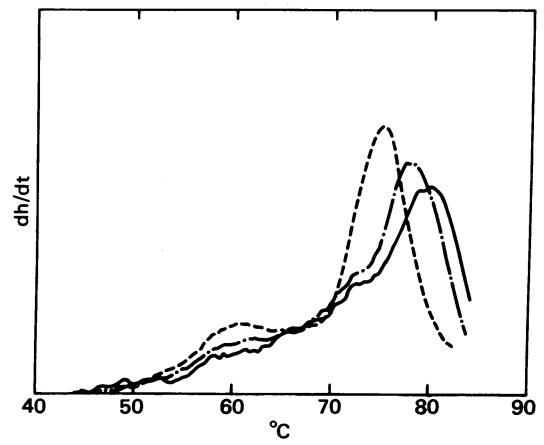

FIG. 2. Thermal denaturation of core particles and compact oligomers. Shown are derivative hyperchromicity curves (dh/dt) for the core particle (- - -), compact dimer (–·–), and compact trimer (—) in 1.0 mM cacodylic acid (pH 7.2).

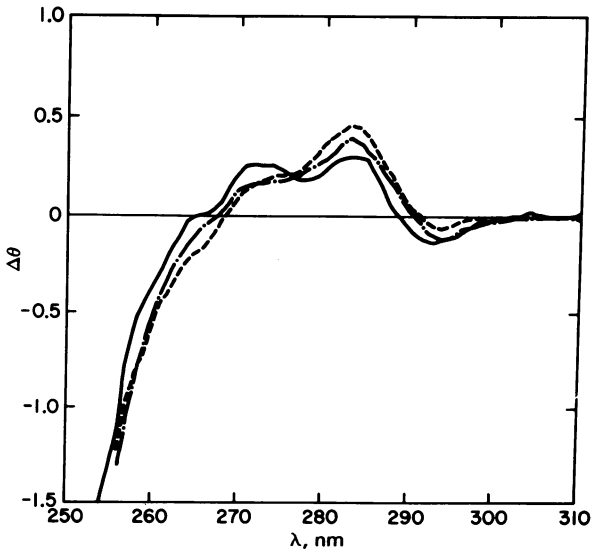

FIG. 3. CD spectra in the near UV of core particles (- - -), compact dimers (–·–), and compact trimers (—).

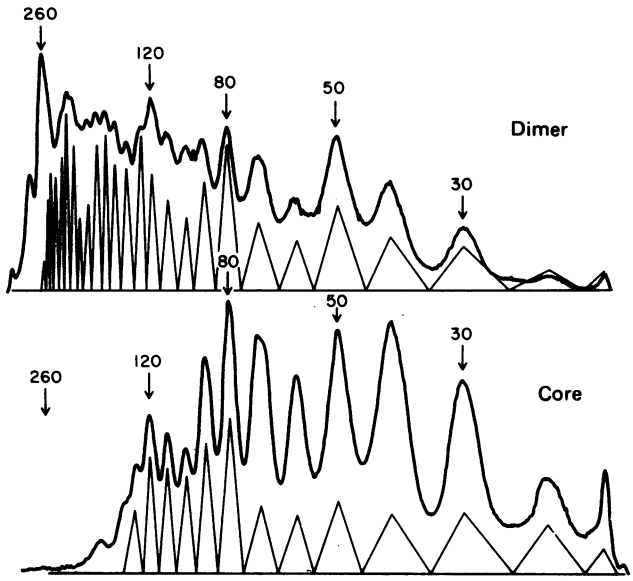

FIG. 4. Gel electrophoresis of single-stranded DNA fragments from DNase I digestion of compact dimers and core particles. The data are shown as scans of ethidium bromide-stained gels. The triangles represent (by height) weight amplitudes of different bands as predicted by the model calculation described in the *Discussion*.

support this model. We have isolated representatives of the first two series—i.e., $(120n + 20)$ and $(130n + 10)$. These particles should be expected to be stable to long nuclease digestion because there is no spacer region susceptible to nuclease attack. The next series, $140n$, was not obtained, possibly because it most likely would possess a region or locus susceptible to micrococcal nuclease and not be stable through the long periods of nuclease digestion used to prepare the particles.

If the $(120n + 20)$ series represents the most compact oligomers in which core stacking is possible, then further shortening of the DNA should lead to steric hinderance by overlap

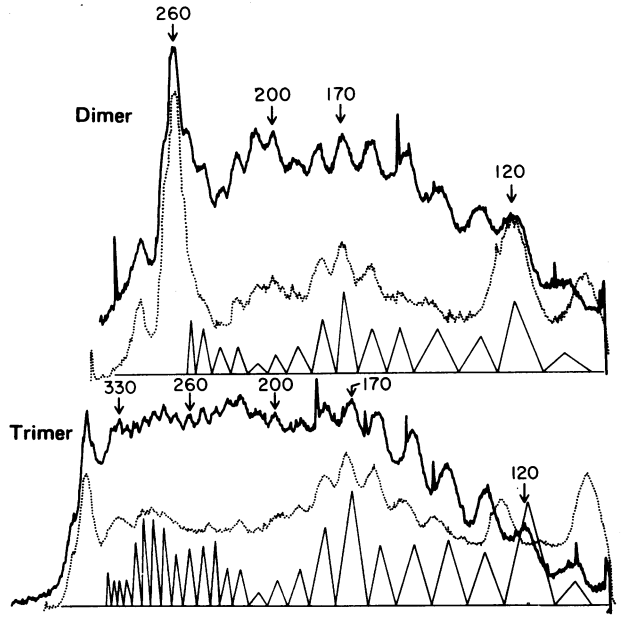

FIG. 5. Gel electrophoresis of single-stranded DNA fragments from a DNase I digestion of compact dimers and trimers end labeled with ^{32}P prior to the digestion. The data are shown as scans of ethidium bromide-stained gels (solid line) or scans of the autoradiogram (dotted line). The triangles represent (by height) amplitudes of different ^{32}P-containing bands as predicted by the model calculations described in the *Discussion*.

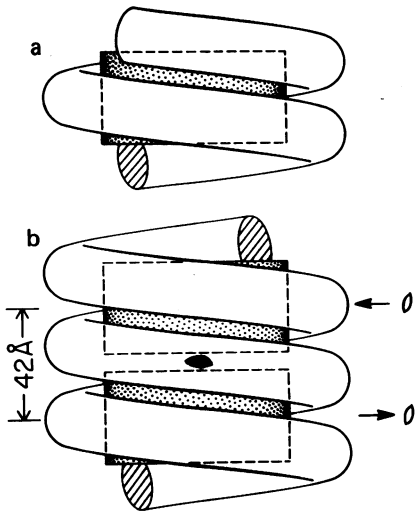

FIG. 6. (*a*) Model of the core particle based on the data of Finch *et al.* (18) and Pardon *et al.* (19). The protein core is shown as a cylinder (stippled) about 70 Å in diameter and 35 Å high. The 140-bp DNA is wound about this in 1.75 turns in a 28-Å pitch helix. (*b*) Model of compact dimer. The 260 bp DNA are wound about two protein cores with coincident cylinder axes. The (pseudo) dyad axes (0) of two core particles are rotated 180° with respect to one another about the cylinder axis. The particle thus created has a (pseudo) dyad axis (⬬) perpendicular to both of the above axes. Other models with the protein cores offset with respect to each other cannot be excluded.

of protein cores. Recently, Klevan and Crothers (2) have isolated a "compact" dimer with 240 bp of DNA. Evidence from electric dichroism studies indicates that these dimer particles are somewhat asymmetric, and a "slipped disk" structure has been proposed (25). We do not have rotational diffusion data for our particles. However, we have calculated the expected sedimentation coefficients for our oligomer particles by using a linear "contacting bead" model (see ref. 26, pp. 234–245) and correcting for the fact that the molecular weights are not exact multiples of the monomer weight. The results are given in Table 1. Although such calculations are both approximate and rather insensitive, the values are certainly not in contradiction to our model. However, we cannot dismiss an asymmetric structure for our particles with the available data.

We now turn to interpretation of the melting and CD data. In a recent study in this laboratory, Weischet *et al.* (6) found that 40 bp of the DNA in the core particle thermally denature in a melting transition 15° below the main transition. They proposed that the ends of the DNA were involved in the premelt. If we apply this to our model of compact oligomers we would expect to see the *percentage* of the premelt decrease because the 40 bp of DNA at the ends would make a smaller fraction of the total. The data in Table 1 indicate that the percentage in the premelt does go down, although not to the exact amount predicted.

The increase in temperature of the main transition can also be explained in light of the finding that the denaturation of the protein in the core particle precedes the main DNA transition by a few degrees (6). If the denaturation of the protein core initiates the denaturation of the DNA, then stabilization of the protein should increase the transition temperature of the DNA. In our model of the compact oligomers, the protein cores are in close proximity and could possibly stabilize one another, thereby raising the transition temperature of the DNA. The compact trimer would be further stabilized by virtue of the fact that the center histone octamer has octamers on either side.

The changes we see in the CD spectra (Fig. 3) can be inter-

preted in a number of ways. For example, the differences between core and compact dimer and trimer could be interpreted as a greater amount of overwound DNA in the dimer and trimer particles (27, 28). An argument similar to the one proposed to explain the thermal denaturation could be used here if it were assumed that the DNA ends were more like the solution form of DNA. The compact oligomers, with proportionally less "end" DNA, would have less ellipticity.

Another possibility is that the supercoils of duplex DNA are close enough in the nucleosome to interact and perturb the CD spectra (29). Such an interaction would have an even greater effect in the larger oligomers because of the greater amount of tight coiling. However, at this time we have no conclusive evidence for this or any other model.

We have attempted to predict the DNase I cutting pattern of the compact dimer and trimer by using the model of Finch *et al.* (18) to predict nuclease-susceptible and -resistant sites. Attacks at 10, 20, 40, 50, 90, 100, 120, and 130 bp along the 140-bp core DNA are assumed to occur with equal frequency with no cutting allowed at the other sites. Because the histone octamer dictates the sites of nuclease cutting, we have extended this cutting model to 260 or 270 bp for the dimer and to 380–400 bp in the trimer. The cutting is assumed to be symmetrical from the two ends, in agreement with the postulated dyad symmetry.

By allowing only one or two DNase I cuts per particle and by tallying up all possible combinations of products, we have generated the expected frequency distributions for the fragments from the core particle, dimer, and trimer. In Fig. 4 the theoretical weight average distributions for the core particle and dimer are presented with the experimental data. It is interesting that our theoretical ladder, with all its simple and perhaps naive assumptions, fits the data so well. The only serious discrepancy is prediction of a major band at 130 b instead of 120 b as observed.

Similar comparisons for the trimer (not shown) indicate that we have correctly predicted the basic curve shape but not necessarily the exact peaks of maximum intensity. With the large particles the envelope of peaks is relatively uniform, and strong conclusions are hard to make.

We have also derived a theoretical pattern for the DNA fragments containing the 5′-end label. Instead of using an "all or nothing" cutting model as we did to predict the total DNase I ladder pattern, we assigned DNase I cutting frequencies to each 10-bp site, starting from the 5′ end of the DNA. The results from the core particle, which were the same as obtained by Simpson and Whitlock (9), were used to predict the cutting frequency along the 260 or 270 bp of DNA in the dimer and the 380–400 bp in the trimer (Fig. 5). We have no explanation for the inexact alignment of the ethidium bromide scan and autoradiogram at 120 b in the trimer. Again, the theory bears marked likeness to the scan of the autoradiogram, lending credibility to the model. These results are of special importance because they virtually rule out models for the compact oligomers that involve closely spaced 140-bp core particles with additional end trimming. Such particles should show prominent bands at other positions.

The physiological significance of these structures is not known at the present time. Preliminary evidence from our laboratory (W. O. Weischet, J. Allen, and K. E. Van Holde, unpublished data) indicates the compact oligomers may be a product of nucleosomal sliding in the 0.6 M NaCl used in preparation of the H1-depleted chromatin. We do not know at this time if all or only a subset of the nucleosomes can slide to form compact oligomers.

Even if the most compact structures are not observed in chromatin *in vivo*, the symmetry arguments for quantization of inserted DNA are still valid. In relatively compact chromatin, parallel stacking of cores may be of importance, and this will impose restrictions on insertion lengths.

Note Added in Proof. A recent paper [Steinmetz, M., Streeck, R. & Zachan, H. G. (1978) *Eur. J. Biochem.* **83**, 615–628] provides strong evidence for nucleosome core sliding in high salt.

We thank Drs. D. Crothers and G. Fasman for furnishing us preprints of papers. We are very much obligated to Dr. R. T. Kovacic for furnishing us data on PM2 restriction fragment sizes. We thank Dr. Caroline Tahourdin for assistance in the ^{32}P end labeling. Discussions with Drs. Wolfgang Weischet and Dennis Lohr were very useful. This research was supported by grants from the American Cancer Society, National Science Foundation, and U.S. Public Health Service. K.V.H. acknowledges a Research Professorship from the American Cancer Society.

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