Periodicity of Exonuclease III Digestion of Chromatin and the Pitch of Deoxyribonucleic Acid on the Nucleosome†

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ABSTRACT: Exonuclease III has previously been shown to pause about every 10 nucleotides along the 3′ strands while it invades the nucleosome core. Here, the exact periodicity of this digestion, i.e., the spacing of the pauses, was determined. Results showed that the exonuclease digests the first 20 nucleotides at the edge of the nucleosome core with a periodicity of approximately 11 nucleotides; in contrast, DNA closer to the center of the particle is digested with a smaller periodicity of about 10 nucleotides. These figures differ from the known periodicity of DNase I digestion, approximately 10 and 10.5 nucleotides at the edge and in the center of the nucleosome, respectively. Moreover, as shown by sedimentations in sucrose gradients, the structure of the nucleosome does not appear to be significantly altered by the gradual destruction of its DNA moiety by the exonuclease. Such stability of the nucleosome, along with other complementary observations, indicates that the transition in the digestion periodicity of the exonuclease may not be the consequence of a structural rearrangement of the particle upon trimming. This transition may rather be ascribed to the properties of the native nucleosome and to the intrinsic mechanism of action of the enzyme. Finally, evidence is presented which suggests that the exonuclease 10-nucleotide periodicity of digestion of the inner region of the nucleosome reflects a 10 base pair/turn pitch of the DNA in that region.

When Noll (1974) first described the digestion of DNA on the nucleosome by DNase I, there was little doubt that the 10-nucleotide periodicity reported for this digestion reflected a 10 base pairs (bp)[1]/turn helical periodicity of nucleosomal DNA (Finch et al., 1977). Indeed, this value was identical with that previously found by X-ray diffraction of DNA in fibers. However, the upward revision of this digestion periodicity to about 10.4 nucleotides (Prunell et al., 1979; Lutter, 1979; Simpson & Kunzler, 1979), together with the determination of the helical periodicity of DNA free in solution, about 10.6 bp/turn (Wang, 1978, 1979; Peck & Wang, 1981; Strauss et al., 1981; Rhodes & Klug, 1981), later raised the question of the actual pitch of the DNA on the nucleosome.

This question was also pertaining to a basic paradox: even though DNA is coiled into two negative superturns around the histone core (Finch et al., 1977), there appears to be only about one negative topological turn of DNA per nucleosome (Germond et al., 1975). With respect to the pitch of nucleosomal DNA, there are essentially two possibilities. First, the pitch might be equal to the digestion periodicity, i.e., close to the pitch of DNA free in solution. Second, the association of the DNA with the histone core may cause an overwinding of the double helix, decreasing its helical periodicity from 10.6 bp/turn in solution to approximately 10 bp/turn (Finch et al., 1977). This decrease would explain the topological discrepancy without need for further stipulations on the path of linker DNA (Finch et al., 1977), as would be required if the pitch of nucleosomal DNA was closer to its digestion periodicity (Worcel et al., 1981).

Different values of the pitch of nucleosomal DNA have also been shown to impose different restraints on the arrangement of nucleosomes in the chromatin fiber, regardless of the topological problem (Strauss & Prunell, 1983). This arises from the property of internucleosomal lengths in at least a subset of the nucleosomes being integral multiples of the helical repeat of the DNA plus about 5 bp. As it turned out, neighbor nucleosomes should be coplanar and packed in a side by side fashion if the pitch of nucleosomal DNA is close to its digestion periodicity. With a pitch closer to 10 bp/turn, in contrast, nucleosomes could be stacked on top of one another with the DNA forming a regular left-handed superhelix (Strauss & Prunell, 1983). It is noteworthy that the stack model, with one negative topological superturn of DNA per nucleosome, solves the paradox while the side by side arrangement, with two negative topological superturns per nucleosome, leaves the paradox open.

It appeared later that helical and cleavage periodicities of DNA on the nucleosome did not have to be the same, making plausible the above hypothesis of a decrease in the pitch of nucleosomal DNA as compared to that of free DNA. In fact, if there was mutual sterical hindrance between the two superturns on the nucleosome or, in other words, if the upper superturn was more accessible from above and the lower superturn more accessible from below, the digestion periodicity would be larger than the helical periodicity (Prunell et al., 1979). Along this line, Klug & Lutter (1981) have since argued, on the basis of a model for the three-dimensional map of the cleavage sites on the nucleosome, that a value close to 10 bp/turn for the pitch of the DNA was the most sterically consistent.

The present work essentially describes the measurement of the digestion periodicity of exo III (Richardson et al., 1964). As previously shown, this exonuclease has the ability to invade the nucleosome, pausing every approximately 10 nucleotides along the 3′ strands. After the DNA is extracted, digested with S1 nuclease, and electrophoresed in a gel, a specific ladder of fragments spaced by about 10 bp appears (Prunell & Kornberg, 1977, 1978; Riley & Weintraub, 1978). Although these fragments resemble those released from chromatin by DNase I, they are basically different. DNase I fragments arise from nicking of the DNA at regular intervals along both strands and reveal sites of exposure of the DNA; in contrast,

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[1] Abbreviations: bp, base pairs; exo III, exonuclease III; PMSF, phenylmethanesulfonyl fluoride; EDTA, ethylenediaminetetraacetate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; Tris, tris(hydroxymethyl)aminomethane.

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exo III fragments correspond to the products of exonuclease digestion of individual nucleosomes and reflect the existence of periodic blocks against the progression of the enzyme. These different mechanisms of action suggest that exo III and DNase I may recognize different features of the nucleosome architecture, which may have two consequences. First, digestion periodicities of the two enzymes may be different, confirming that the pitch of nucleosomal DNA may not necessarily be reflected by its digestion periodicity. Second, digestion periodicity of the exonuclease might be closer to the actual pitch of the DNA than digestion periodicity of the DNase.

A basic problem with the use of an exonuclease, however, is that an exonuclease destroys the DNA, i.e., the structure being examined, in contrast to a DNase which can be allowed to inflict only a few cuts on each particle. Details revealed by exo III trimming might, therefore, reflect alterations rather than properties of the native structure. This problem prompted a correlative investigation of the stability of nucleosomes upon trimming.

Materials and Methods

Materials. Rats used in this study were Wistar males. Micrococcal and S1 nucleases were purchased from Sigma, and T4 polynucleotide kinase was from New England Biolabs. [γ-^{32}P]ATP was purchased from New England Nuclear and Amersham. Exo III obtained from several sources, including New England Biolabs and Bethesda Research Laboratories, yielded identical results.

Chromatin Preparation. Rat liver nuclei were prepared and digested with micrococcal nuclease as previously described (Prunell & Kornberg, 1977). H1-depleted nucleosome monomers were purified by sedimentation through isokinetic sucrose gradients containing 1 mM EDTA, 1 mM sodium bisulfite, pH 7, and 0.55 M NaCl, dialyzed in the cold against 1 mM EDTA, 1 mM sodium bisulfite, pH 7, and 0.2 mM PMSF, concentrated against solid sucrose, dialyzed again against 1 mM Tris-HCl, 0.1 mM EDTA, pH 7.5, and 0.2 mM PMSF at 4 °C, and stored on ice.

Preparation of Core Particles. Core particles were prepared essentially as described by Lutter (1978). In outline, nuclei were briefly digested with micrococcal nuclease, and soluble, long chromatin was stripped of H1 by filtration through Sepharose 4B in the presence of 0.5 M NaCl. H1-depleted chromatin was subsequently digested with micrococcal nuclease into core particles. Core particles were purified by filtration through Sepharose 6B, dialyzed against 10 mM Tris-HCl, 0.05 mM EDTA, pH 7.5, and 1 mM 2-mercaptoethanol, and stored on ice.

Gel Electrophoresis. Electrophoresis of DNA was performed in polyacrylamide slab gels containing 98% formamide (Maniatis et al., 1975). Gel dimensions were 16 × 0.15 × 30 cm, except where otherwise stated. DNA fragments were calibrated by using size markers of known sequences which were obtained from recombinant plasmids carrying the *Escherichia coli lac* region. These markers have previously been described (Prunell et al., 1979).

Other Methods. Dephosphorylation of DNA and end labeling with [γ-^{32}P]ATP and polynucleotide kinase were performed as described by Prunell et al. (1979). DNA was digested with S1 nuclease according to Strauss & Prunell (1982).

Results

Hydrodynamic Properties of Exo III Digested Nucleosomes. H1-depleted nucleosome monomers were labeled at their DNA 5′ ends with ^{32}P and digested with exo III. The extent of digestion was monitored by gel electrophoresis of the DNAs extracted from the digests, before and after S1 nuclease treatment. Figure 1 shows an autoradiogram of the gel and its photograph after staining. As seen in the figure, S1 treatment reveals bands which extend from about 140 nucleotides down to about 40 nucleotides, and below.

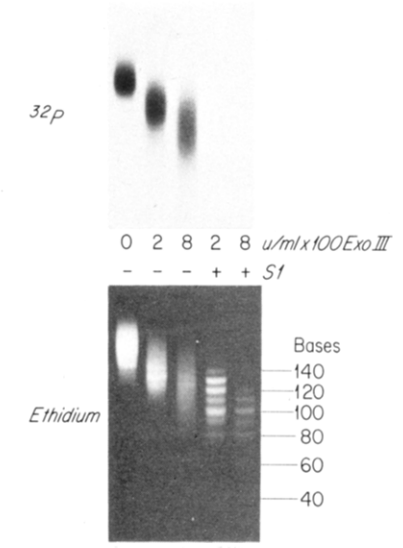

FIGURE 1: Hydrodynamic properties of exo III trimmed monomers. Control of the extent of digestion. H1-depleted nucleosome monomers, at a DNA concentration of 40 μg/mL in 50 mM Tris-HCl, pH 8.5, 5 mM $MgCl_2$, and 15 mM 2-mercaptoethanol, were labeled by incubation with [^{32}P]ATP and polynucleotide kinase (0.5 unit/μg of DNA) for 1 h at 37 °C and purified by sedimentation in isokinetic sucrose gradients. A 10-fold excess of unlabeled H1-depleted monomers was added to a DNA concentration of 80 μg/mL. The mixture, in 10 mM sodium phosphate, 1 mM sodium bisulfite, 0.2 mM EGTA, pH 7.4, 1 mM 2-mercaptoethanol, and 0.5 mM $MgCl_2$, was incubated for 1 h at 37 °C with the indicated concentrations of exo III, and digestion was terminated by adding EDTA to 1 mM. DNAs extracted from the digests were electrophoresed in a 98% formamide–8% polyacrylamide gel (16 × 0.15 × 18 cm) before or after treatment with S1 nuclease (30 units/mL; 30 min at 37 °C). Both an autoradiogram and a photograph of the stained gel are shown.

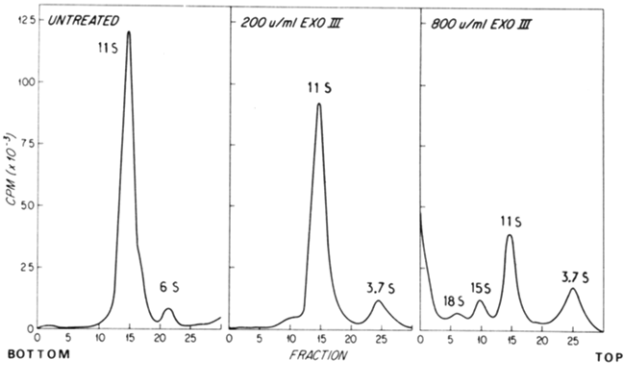

FIGURE 2: Hydrodynamic properties of exo III trimmed monomers. Sedimentation profiles. Aliquots of exo III digested nucleosomes described in the legend of Figure 1 were layered on top of isokinetic sucrose gradients containing 1 mM EDTA and 1 mM sodium bisulfite, pH 7.0. Centrifugation was carried out at 200000g for 16 h at 4 °C. Fractions were collected and counted.

Figure 2 shows profiles obtained upon sedimentation of the above particles in isokinetic sucrose gradients. Two preliminary remarks can be made. First, the lack of nonsedimenting counts at the top of the gradients shows that the reported 5′-clipping activity of exo III on chromatin (Riley & Weintraub, 1978) does not occur in these preparations. Second, the main peak at about 11 S decreases in amount as digestion proceeds but keeps precisely the same position in the gradients and the same width. The 11S peak, however, contains particles

similar to those of the corresponding total digest, as shown by gel electrophoresis of DNA extracted from the peak (data not shown). In other words, the sedimentation coefficient of a particle remains constant despite the loss, in the most digested sample, of about 30% of its DNA in weight. It can, therefore, be concluded that no gross alteration of its structure occurs upon trimming. Moreover, the constancy observed in the sedimentation coefficient of the particle should actually result from a compensation of its loss of weight by a decrease in its frictional coefficient, as if 5′ single-stranded tails remain bound to it.

Two other peaks in the profiles (Figure 2) are of interest. The first one, at about 6 S, is present in the undigested material while the second one, at about 3.7 S, appears upon trimming. The 6S peak cosediments with DNA extracted from undigested nucleosomes (data not shown), suggesting that it contains DNA released from some particles during preparation of the sample. The 3.7S peak sediments at about the same position as naked DNA extracted from starting nucleosomes and digested to completion with exo III (data not shown), suggesting that DNA in nucleosomes can also be digested to completion by the exonuclease.

The decrease in the amount of the 11S peak correlates with the appearance of new peaks at about 15 and 18 S, indicating that they are formed by aggregates of two and three particles, respectively. Aggregates of more than four or five particles pellet at the bottom of the gradient. Such an aggregation may be induced by the weakening of the repulsion forces between particles as a consequence of the decrease in their net negative charge. However, the fact that this aggregation involves all particles, not only the most digested particles, suggests that the main factor may be the exposure, by exo III digestion, of histone DNA-binding sites which interact with DNA of other nucleosomes. This aggregation probably blocks further digestion, which explains why it is not possible to trim nucleosomes much further than shown in Figure 1, and also why the 3.7S peak material is generated only in limited amounts. Aggregation and subsequent blocking of digestion have also been observed when chromatin is degraded with micrococcal nuclease (Kornberg, 1977).

In conclusion, the structure of nucleosomes does not appear to be significantly altered upon extensive trimming with the exonuclease. Moreover, it appears that exo III has the ability to degrade nucleosomal DNA to completion, although digestion is generally blocked before that stage because of an aggregation of the particles.

Periodicity of Exo III Digestion. H1-depleted nucleosomes were digested with exo III to two different levels. DNAs were extracted, digested with S1 nuclease, and labeled at the ends with ^{32}P. Fragments were then calibrated by comparison with size markers of known sequences by gel electrophoresis in a formamide-containing polyacrylamide gel. Exo III + S1 fragments and marker fragments were electrophoresed as a mixture in one lane of the gel or separately in adjacent lanes (Figure 3), so that a densitometric trace of the mixture could be used to align traces of the other lanes (see below). These strongly denaturing conditions of electrophoresis are known to avoid any nucleotide sequence dependence of the mobilities of the fragments (Prunell et al., 1979), as shown by the sharp bands formed in Figure 3 by the two strands of the marker fragments. Size markers have previously been cross-checked by comparison with a large number of other markers and shown to lead to reliable estimates of fragment lengths (Prunell et al., 1979). The autoradiogram of the gel (Figure 3) shows well-defined bands which extend from about 140 nucleotides

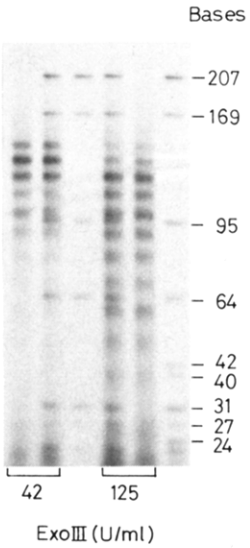

FIGURE 3: Periodicity of exo III digestion. Fractionation of exo III + S1 digests of nucleosome monomers. H1-depleted nucleosome monomers, at a DNA concentration of 50 μg/mL in 10 mM Tris-HCl, 0.2 mM EGTA, pH 7.5, 1 mM 2-mercaptoethanol, and 0.5 mM $MgCl_2$, were digested with the indicated concentrations of exo III for 30 min at 37 °C. Digestions were stopped by addition of EDTA to 1 mM. DNAs were extracted from the digests, incubated with 30 units/mL S1 nuclease for 30 min at 37 °C, and subsequently dephosphorylated and end labeled with ^{32}P by using polynucleotide kinase. DNAs were electrophoresed in a 98% formamide–8% polyacrylamide gel along with size markers of known sequences (see Materials and Methods), either mixed or separated. An autoradiogram of the gel is shown.

Table I: Periodicity of Exo III Digestion of Nucleosomes[a]

band no. (*n*)	length (nucleotides)	
	early digest	late digest
14	142.7	143.4
13	132.1	132.3
12	121.2	121.1
11	110.4	110.4
10	99.4	99.3
9	89.5	89.5
8	79.0	79.2
7	68.7	68.9
6	59.0	59.3
5		49.1
4		39.0

[a] Lengths of DNA in exo III + S1 nuclease trimmed particles were measured in the gel of Figure 3 and other similar gels by comparison with size markers of known sequences, as described in Figure 4. The average obtained from three (earlier digest) and four (later digest) different gels is given. The precision of the measurements varies between ±0.2% and 0.4% (see error bars in Figure 5).

in both digests down to 40 nucleotides in the later digest. More indistinct bands are, however, present below that size in both digests; the largest of these bands is centered between 25 and 26 nucleotides (see Figure 3). Lengths were measured from densitometric traces of the autoradiogram (Figure 4) and are listed in Table I. As shown in Table I and in Figure 4, these lengths do not depend on the amount of exo III. The excess of these lengths over 10*n* nucleotides is plotted in Figure 5 as a function of the band number (*n*). Figure 5 shows two domains separated by an abrupt transition at band 10. In the first domain (bands 4–10), the average spacing between the pauses, i.e., the periodicity of exo III digestion, is equal to about 10 nucleotides (10.06 nucleotides, as calculated by a least-squares procedure), and the line joining the points is almost parallel to the abscissa. In the second domain (bands 10–14),

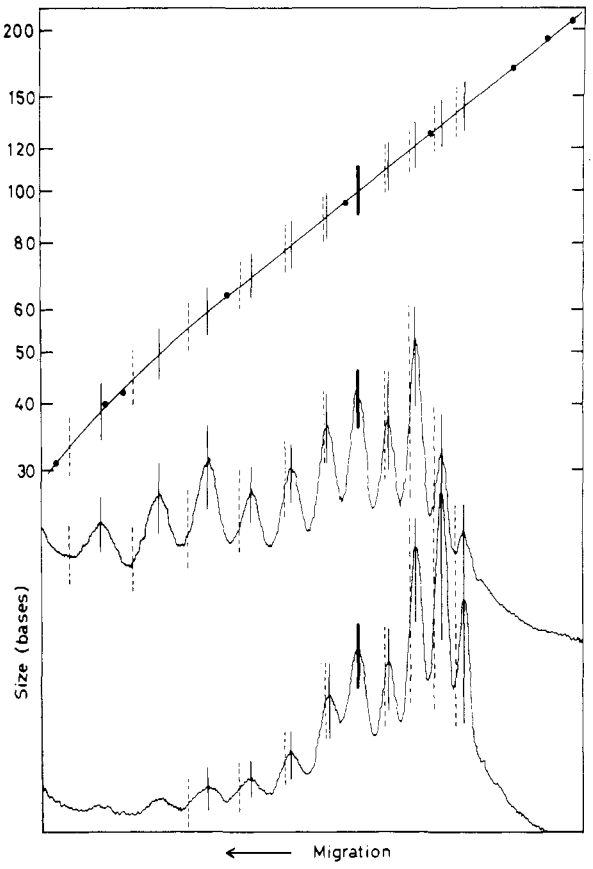

FIGURE 4: Size determination of exo III bands. Densitometer traces of lanes in Figure 3 which contain the two exo III digests (bottom) and the marker fragments were aligned. Sizes and distances of migration of the marker fragments (filled circles) were used to construct a calibration curve (top) from which the sizes of the exo III bands were derived. Data corresponding to the 192- and 128-nucleotide markers were taken from Figure 9. The distances of migration of the exo III fragments (solid vertical lines) are contrasted with those expected (dashed vertical lines) if the spacing between the bands, i.e., the digestion periodicity, was equal to 10 nucleotides to the right of band 10 (thick vertical bar) and to 11 nucleotides to the left.

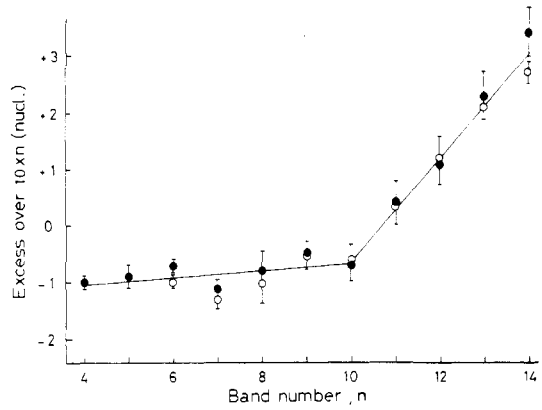

FIGURE 5: Periodicity of exo III digestion of nucleosome monomers. Data are taken from Table I. Error bars show the standard deviation of the measurements. (○) Earlier digest; (●) later digest.

the periodicity of the digestion is larger, being close to 11 nucleotides (10.9 nucleotides). The precision of the measurements is better than ±0.5%, i.e., ±0.5 nucleotide for the 100-nucleotide band. Such precision can be achieved despite the width of the bands being close to five nucleotides because the center of broad but symmetrical bands can be accurately determined. For example, the centers of the bands are clearly

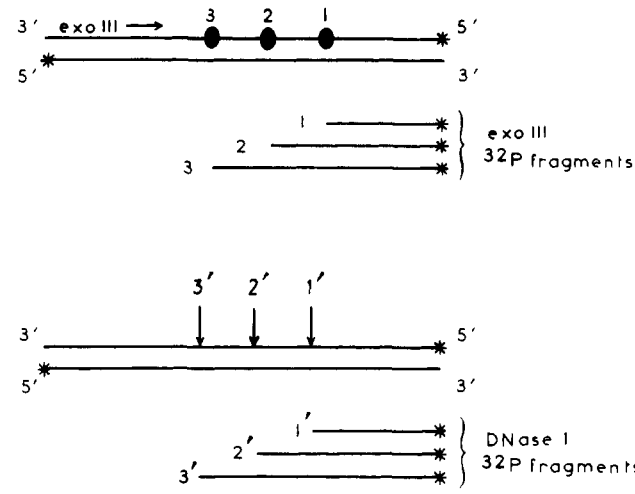

FIGURE 6: Mapping of exo III pauses in the core particle. Diagram of the experiment. ^{32}P-Labeled core particles are digested with exo III or DNase I. Exo III pauses (●) and DNase I cuts (↓), respectively, are superimposed on the same strand for convenience.

distinguishable from those expected if the periodicity of the digestion was unique and equal to 10 or 11 nucleotides, respectively, throughout the nucleosome (Figure 4).

Mapping of Exo III Pauses in the Core Particle. In order to investigate whether the shift observed in the digestion periodicity of exo III occurs at a specific position in the nucleosome, pauses were mapped as depicted in the diagram of Figure 6. Core particles were produced by micrococcal nuclease digestion of chromatin, end labeled with ^{32}P and digested with exo III. The lengths of single-stranded fragments designated by 1, 2, and 3 in Figure 6 then reflect the positions of the pauses relative to the 5′ termini. These particles may also be digested with DNase I, in which case length differences between single-stranded fragments 1′, 2′, and 3′ and 1, 2, and 3 (see Figure 6) give the distances separating the pauses from their neighboring DNase I sites. In the actual experiment, and in order to ensure more flexibility, exo III and DNase I digestions were performed instead on unlabeled core particles, and DNAs were end labeled with polynucleotide kinase after extraction from the digests. These two procedures were found to give the same results (data not shown), as expected from the fact that no additional 5′-OH end is produced by exo III or by DNase I digestion.

Lengths of the fragments were measured by electrophoresis in gels similar to those described above. Autoradiograms of these gels are shown in Figure 7. The two gels which are presented correspond to digestions in different buffers. Ninety-nucleotide bands appear to be the smallest well-defined bands in both cases, although these bands can only be seen upon overexposure of the gel (not shown) when digestion is performed in the higher ionic strength buffer. DNase I patterns, on the other hand, are typical, with strong 50- and 40-nucleotide bands and weak 110-, 80-, 60-, and 30-nucleotide bands (Simpson & Whitlock, 1976; Noll, 1977; Lutter, 1978). Interestingly, activation of both enzymes is found in the higher as compared to the lower ionic strength buffer. Activation of exo III is observed, however, only at the lower level of digestion (compare 42 and 125 units/mL fractions); at the higher level of digestion, in contrast, exo III appears to be inactivated (compare 525 and 625 units/mL fractions). This effect may originate, at least in part, from enhancement of trimming-induced aggregation of nucleosomes (see above) at the higher ionic strength, as suggested by the faster increase in the turbidity of the incubation mixture under these con-

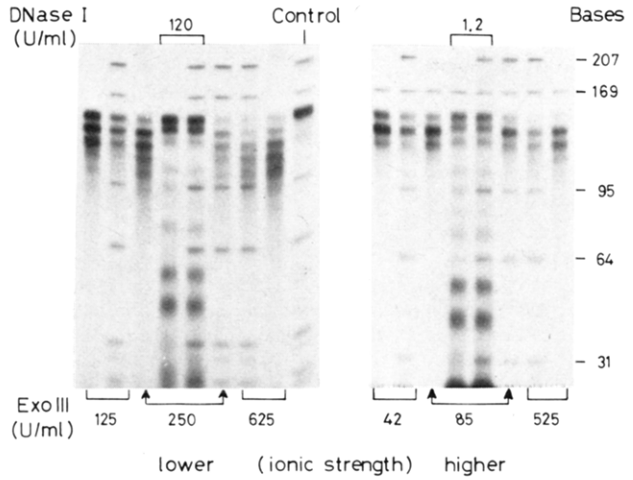

FIGURE 7: Mapping of exo III pauses in the core particle. Fractionation of exo III and DNase I digests. Core particles were prepared as described under Materials and Methods and digested at 37 °C with the indicated concentrations of exo III (for 30 min) or DNase I (for 15 min). The following two different digestion buffers were used: 10 mM Tris-HCl, 0.2 mM EGTA, pH 7.5, 1 mM 2-mercaptoethanol, and 0.5 mM $MgCl_2$ (left panel); 50 mM Tris-HCl, pH 7.5, 5 mM 2-mercaptoethanol, and 5 mM $MgCl_2$ (right panel). Digestions were stopped by addition of EDTA to twice the concentration of $MgCl_2$. DNAs were extracted, end labeled with ^{32}P by using polynucleotide kinase, and electrophoresed in 98% formamide–8% polyacrylamide gels, along with DNA from starting core particles and size markers. Autoradiograms are shown.

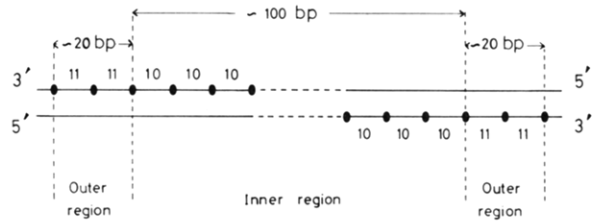

FIGURE 8: Scheme of the periodicity of digestion of 3′ ends of nucleosomal DNA by exo III. (●) Exo III pause. Data are taken from Table II, except data for the first digestion step (11 nucleotides) and the last (10 nucleotides), respectively, which were inferred from the periodicities observed in Figure 5 (see Results).

ditions (data not shown). Upon measurement, the sizes of exo III bands were found not to depend on the extent of digestion, with the exception of band 14 whose size showed a significant increase upon digestion; this effect appears to result from a resistance of some nucleosomes to digestion, which may be due, for example, to the existence of 3′ protruding ends in these particles. Sizes of exo III bands were also found to be virtually identical under both digestion conditions. These sizes are listed in Table II, together with the periodicity of the digestion, i.e., the size increment between adjacent bands. Table II shows that the 11- to 10-nucleotide shift in the periodicity of digestion now occurs at band 12, indicating that the first 20 nucleotides at the edges of the nucleosome are digested with the larger periodicity while DNA closer to the center is digested with the smaller periodicity. This mode of digestion of nucleosomal DNA is outlined in Figure 8. The large apparent periodicity of digestion of the first 10 nucleotides, which results from the large size of band 14 (see Table II), appears to be artifactual. Indeed, two factors contribute to increase the size of this band. The first one is the apparent resistance of some particles to exo III digestion (see above). The second factor is the contribution to the size of the band of starting particles waiting to be digested; this contribution is expected to be more significant at a lower level of digestion.

Table II: Mapping of Exo III Pauses in the Nucleosome Core [a]

	nucleotides			
band no.	exo III [b]	periodicity [c]	DNase I [d]	DNase I − exo III
14	145.5–146.4			
		⩾11		
13	134.4		136.5	2.1
		11.0		
12	123.4		126.7	3.3
		9.8		
11	113.6			
		9.8		
10	103.8		105.9	2.1
9			95.0	

[a] Fragments released from core particles by exo III and DNase I digestions (see scheme of Figure 6) were calibrated in the gels of Figure 7 and other similar gels by comparison with size markers of known sequences, as described in Figure 4. [b] Sizes of exo III bands were found to be independent of the extent of digestion and of the ionic strength of the digestion buffer (see Figure 7), with the exception of band 14 whose size appeared to increase upon digestion. Exo III band 9 was too indistinct for its size to be measured with accuracy. The average of the data obtained from four different gels (two for each digestion buffer) is given. The precision of the measurements is about ±0.5%. [c] The periodicity of exo III digestion was obtained by subtracting the size of adjacent bands. [d] Lengths of DNase I fragments were found to be virtually identical with those reported by Lutter (1979).

The length of DNA in starting core particles and also the length of the fragments generated by DNase I under both digestion conditions were found to be very similar to the lengths reported by Lutter (1979). DNase I fragments are always longer than exo III fragments (Table II), indicating that exo III pauses on the 3′ strands are located beyond DNase I cleavage sites (see Figure 6). Their length difference reaches a maximum of more than three nucleotides at band 12 (Table II), i.e., at the position where the shift in the exo III digestion periodicity is observed. Interestingly, this position in the nucleosome also appears peculiar with respect to the stagger between DNase I cleavage sites on opposite strands. Indeed, a three-nucleotide stagger is observed at this position, against two nucleotides at all other positions (Lutter, 1979).

The shift in the digestion periodicity previously observed at about 100 bp in the exo III + S1 patterns (Figure 5) is then likely to result from the present shift at band 12, since the removal of 20 nucleotides at each 3′ end of the core particle leaves 100 bp of double-stranded DNA (see Figure 8). To confirm this expectation, DNA from the most digested exo III core particles (Figure 7; 625 units/mL) was further digested with S1 nuclease at two different concentrations and terminally labeled with ^{32}P. Sizes of the resulting fragments were measured by gel electrophoresis as described above. The autoradiogram of the gel (Figure 9) shows bands extending from about 120 nucleotides down to about 40 nucleotides. Smaller and more indistinct bands are present, as already observed in Figure 3. Bands corresponding to the lower level of digestion with S1 nuclease were found to contain fragments of virtually the same lengths as those listed in Table I (data not shown). In contrast, the lengths of the fragments corresponding to the higher level of S1 nuclease digestion were all decreased by approximately one nucleotide (data not shown), indicating that the transition in the digestion periodicity which is observed in the exo III + S1 patterns is not, as expected, related to S1 nuclease digestion itself.

Discussion

As far as exo III digestion is concerned, this study has revealed two domains in nucleosomal DNA: an outer region corresponding to 20 nucleotides at the edge of the core particle which is digested with a periodicity of approximately 11 nucleotides and an inner region where this periodicity is about

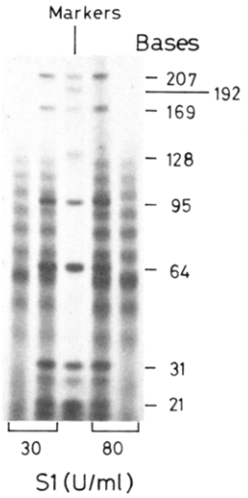

FIGURE 9: Periodicity of exo III digestion. Fractionation of exo III + S1 digests of nucleosome cores. Portions of the DNA from exo III digested core particles, which is shown in Figure 7 (625 units/mL), were digested with the indicated concentrations of S1 nuclease at 37 °C for 30 min. DNAs were subsequently dephosphorylated, end labeled with ^{32}P by using polynucleotide kinase, and electrophoresed in a 98% formamide–8% polyacrylamide gel along with size markers which were run separated or mixed. An autoradiogram is shown.

10 nucleotides (see Figure 8). Before the possible meaning of this observation is discussed in terms of nucleosome structure, several points will be considered.

Stability of Nucleosomes upon Trimming. Even though DNA is gradually destroyed by the exonuclease, the sedimentation coefficient of the particles does not change significantly. Although such an assay is not very sensitive, it suggests that trimmed nucleosomes may otherwise retain most of the features of the native particles. Nucleosomes may actually be stable down to a double-stranded DNA content of 40 bp. First, this length of DNA corresponds to the smallest well-defined band seen in exo III + S1 nuclease patterns from both nucleosomes (Figure 3) and core particles (Figure 9). Second, nucleosomal DNA is digested down to this size with a constant 10-nucleotide periodicity (Figure 5). It is noteworthy that remaining 5′ single-stranded DNA ends may participate in the stability of the trimmed nucleosome. Indeed, single-stranded ends are expected to neutralize part of the positive charges of the histones which would have, otherwise, been exposed by digestion. This view is actually supported by the decrease in the frictional coefficient of the particle observed upon trimming, which implies that single-stranded ends remain bound to the nucleosome core (see Results).

The presence of smaller and more indistinct bands below the 40-nucleotide band (Figures 3 and 9) reveals, however, that particles can be digested further. It appears that their structure may be disrupted at this stage, as suggested by an abrupt increase of more than three nucleotides in the periodicity of digestion below band 4 (see Results and Table I). [Such a disruption was expected since the histone octamer alone is unstable in the low ionic strength buffer used for digestion (Thomas & Butler, 1977).] As shown in Figure 10, these 40 bp of DNA appear to be located in the middle of the particle; another location of the 40 bp fragment would indeed give rise to well-defined bands below 90 nucleotides in the exo III patterns of Figure 7, and these bands are not observed.

It is interesting to note that such remarkable stability of nucleosomes, together with the ability of a protein to progress along one strand through the path of nucleosomal DNA, may help to explain how polymerases could replicate or transcribe DNA in chromatin without disrupting its structure (Leffak et al., 1977; Gariglio et al., 1979).

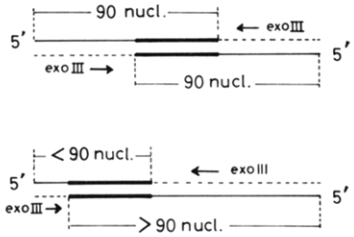

FIGURE 10: Position of the 40 bp limit fragment in the core particle in relation to lengths of single-stranded DNA. Under exo III digestion, 40 bp appear to be a lower limit for the double-stranded DNA content of a stable particle (see Discussion). The 40 bp fragment (thick bars) can be located symmetrically in the particle (top) in which case no single-stranded fragment shorter than 90 nucleotides will be generated. This is in contrast to the case where the fragment is located at another position (bottom).

Synchrony of Exo III Digestion at the Two 3′ Ends. The fact that odd-numbered bands are well represented in the exo III + S1 patterns of Figures 3 and 9 indicates that exo III trims the two ends asynchronously. If digestion of the two ends were rigorously synchronous, then only even-numbered bands at 120, 100, 80, etc. should be present. As a consequence, bands are expected to contain fragments from different regions of the nucleosome. However, the sharp transition in the periodicity of digestion of exo III seen in the exo III + S1 pattern (see Figure 5) suggests that trimming at the two ends is less asynchronous than expected. Indeed, the difference in size between band 10 and band 9 can be equal to 10 nucleotides as observed (Table I and Figure 5) only if band 10 corresponds to a fragment of 100 bp located symmetrically in the nucleosome, as shown in Figure 8. If the 100 bp fragment was located asymmetrically, band 9 could be obtained from band 10 by the removal of either 10 nucleotides at one end or 11 nucleotides at the other (see Figure 8); the difference in size between the two bands could then be equal to 10.5 nucleotides if the two ends were digested equally. It appears, therefore, that band 10, as well as band 14 (this band corresponds to core particles) and band 4 (see above), and probably most of the even-numbered bands in the pattern contain fragments which are mostly located symmetrically in the nucleosome. This view is also supported by the stronger even-numbered bands at 120, 100, and 60 nucleotides in the 125 units/mL digest in Figure 3, and at 100, 60, and 40 nucleotides in the 800 units/mL digest in Figure 1. Moreover, the first band in Figure 9 is at 120 nucleotides, which also suggests that this band originates from the digestion of 10 bp at each end of the core particle and not of 20 bp at a single end; the 60-nucleotide band is also stronger in this figure.

Features Which Specify the Blocks against Exo III Digestion. Since pauses reflect the existence of periodic blocks against the progression of exo III, an interesting question concerns the nature of the features of the nucleosome architecture which specify these blocks. Blocks may actually be provided either by the histones, on which the DNA is bound, or by the adjacent DNA superturn of the two-turn nucleosome, as shown in Figure 11. Whereas the adjacent DNA superturn may play a role during the digestion of the outer region of the nucleosome (Figures 8 and 11), it is clear that histones must be involved after the particles have been digested to less than a single superturn of DNA (Figure 11), i.e., when their DNA content is comprised of between 80 and 40 bp (Figure 8). Interestingly, histones appear to be involved from the very beginning of the digestion of the inner region, despite the fact that the particles still contain more than a single DNA superturn at this point. This can be shown as follows. Let us

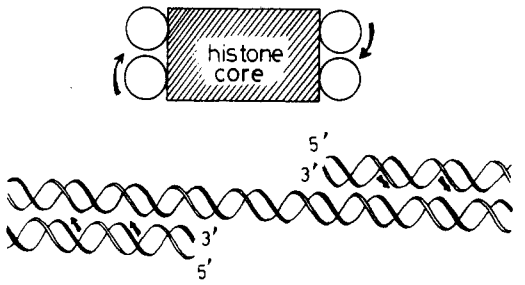

FIGURE 11: Model for exo III digestion of the nucleosome. The figure represents a cross section of the nucleosome in a plane containing the axis of the DNA superhelix (top) and the $1^3/_4$ left-handed turn of this superhelix around the histone core (bottom). These schemes show that exo III (whose direction of progression is represented by the arrows) may impinge upon the adjacent DNA superturn as long as the particle contains more than a single superturn or 80 bp of DNA.

assume that DNA specifies the position of the pauses down to 80 bp. Then, a switch from DNA-specified to histone-specified pauses should occur around 80 bp, i.e., upon complete disappearance of the adjacent DNA superturn. This switch would in turn be expected to displace the pauses in the direction of the digestion by one or more nucleotides, as suggested by Figure 11. In contrast, no such discontinuity is observed around that size in Figure 5. Moreover, the shift which is effectively observed at 100 bp corresponds to a backward displacement of the pauses—the digestion periodicity is decreased—rather than a forward displacement; this suggests that the shift at 100 bp is not related to an early disappearance of the adjacent DNA superturn. In summary, pauses in the inner region of the nucleosome appear to be specified solely by the interaction of the DNA with the histones. The 10-nucleotide periodicity of digestion, therefore, may reflect a 10 bp/turn pitch of the DNA in that region. In contrast, the positions of the pauses on the edges may be influenced by the presence of the adjacent DNA superturn. Consequently, the 11-nucleotide periodicity may not reflect the pitch of the DNA on the edge. It is noteworthy that a similar sterical hindrance may arise during DNase I digestion of DNA throughout the nucleosome (see the introduction). For this reason, the periodicity of DNase I digestion is also not likely to reflect the pitch of nucleosomal DNA.

Does the Nucleosome Undergo a Structural Transition at 100 bp? The 11- to 10-nucleotide transition may reflect a correlative rearrangement of the structural components of the nucleosome which could be triggered when its double-stranded DNA content becomes less than 100 bp. Such a rearrangement, unlike the disruption which presumably occurs at 40 bp (see above), would be subtle since it does not lead to a significant change in the sedimentation coefficient of the particles (see Results). However, several observations argue against this explanation. First, the transition occurs at a position (band 12; see Table II) which already appeared peculiar with respect to the stagger of DNase I sites (see Results), and this latter feature is probably specific to the native particle. Second, gel electrophoresis of DNA from exo III + S1 digests of nucleosomes whose histones had previously been cross-linked into octamers with dithiobis(succinimidyl proprionate) (Lomant's reagent) showed a strong decrease in the intensity of bands 14–11, while bands 10–4 were virtually unaffected (A. Prunell, unpublished results). Such differential accessibility of the histones to the reagent shows that the two domains of the nucleosome which were revealed by exo III digestion also correspond to structurally distinct domains in the intact particle. Moreover, the preservation of the 10-nucleotide periodicity of digestion under these conditions is significant since cross-linking of the histones might prevent a rearrangement of the particle. Third, a trimming-induced structural transition of the nucleosome is expected to be salt dependent since it would result from the unshielding of positive charges on the histones. No such dependence is actually observed (see Figure 7 and Table II).

Conclusions

If, as suggested above, the particle does not rearrange upon trimming, then the simplest explanation for the 10-nucleotide periodicity of digestion is that it reflects the actual pitch of the DNA in the inner region of the nucleosome, about 10 bp/turn. The same conclusion was also drawn from the observation that pauses in the inner region can only be specified by the interaction of the DNA with the histones (see above). It is noteworthy, however, that this conclusion does not apply to the 40 bp central DNA region of the nucleosome (see Figure 8), whose digestion appears to induce the particle to break down (see above). The possibility for DNA at the center of the nucleosome to have a pitch different from 10 bp/turn remains, therefore, open.

In contrast to the case of the 10-nucleotide periodicity, little information on the pitch of the DNA on the edges of the nucleosome can be derived from the 11-nucleotide periodicity observed upon digestion of that region with exo III. As observed above, this uncertainty results from the possibility for the positions of the corresponding pauses to be influenced by the presence of the adjacent DNA superturn in the two-turn nucleosome. However, considering that the particle is more likely to preserve its native structure at this early stage of trimming, it is interesting that exo III and DNase I digest the edge regions with different periodicities, 11 against about 10.2 nucleotides (Lutter, 1979, 1981), respectively. [A larger digestion periodicity of about 10.6 nucleotides is observed with DNase I in the center region, so that the average periodicity throughout the nucleosome is close to 10.4 nucleotides (Lutter, 1979, 1981; Prunell et al., 1979).] This discrepancy supports the notion, mentioned in the introduction, that helical periodicity and digestion periodicity of nucleosomal DNA need not be the same (Prunell et al., 1979). It can be concluded, therefore, that the pitch of DNA on the edges as well as in the center of the nucleosome remains essentially unknown; this pitch may, in particular, be different from 10 bp/turn (see below).

Recent experiments on the reconstitution of chromatin on a recombinant plasmid DNA carrying a 20 bp insert of poly(dA)·poly(dT) have shown that this insert has a marked preference to occupy the two side regions of the nucleosome core. In contrast, the insert was found to be essentially excluded from the very edges of the particle (about 20 bp on each side), as well as from its central approximately 20 bp region (Prunell, 1982). It appears, therefore, that the insert prefers to occupy what has been specified here as the inner region of the nucleosome (Figure 8). In other words, poly(dA)·poly(dT) prefers to occupy a region of the nucleosome where the pitch of the DNA may be identical with its own pitch free in solution (10.1 bp/turn; Peck & Wang, 1981; Strauss et al., 1981; Rhodes & Klug, 1981). This coincidence favors winding constraints over bending constraints (Prunell, 1982) as the reason for the exclusion of the poly(dA)·poly(dT) insert from the edges and from the center of the nucleosome. Indeed, winding constraints will develop in the poly(dA)·poly(dT) double helix and may oppose its positioning in those regions if the pitch of nucleosomal DNA there is, as mentioned above, different from 10 bp/turn. These results appear, therefore, to raise the interesting possibility that the pitch of DNA varies

from the edge to the center of the nucleosome.

Acknowledgments

I thank Dr. Roger Kornberg, in whose laboratory early experiments of this work were carried out.

Registry No. Exo III, 9037-44-9.

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Secondary Structure Assignment for α/β Proteins by a Combinatorial Approach†

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ABSTRACT: We describe an algorithm for assigning the secondary structure of α/β proteins. Turns are identified very accurately (98%) by simultaneously considering hydrophilicity and the ideal spacing of turns throughout the sequence. The segments bounded by these turns are labeled by a pattern-recognition scheme based on the physical properties of α-helices and β-strands, in this class of proteins. Long-range, as well as local, information is incorporated to enhance the quality of the assignments. Although the assignment for any one sequence is not unique, at least one of the assignments bears a close resemblance to the native structure. The algorithm successfully divides protein sequences into two classes: α/β and non-α/β. The accuracy of the secondary-structure assignments in the α/β class is sufficient to provide useful input for tertiary-structure assignments.

Protein tertiary structure is specified by the primary amino acid sequence (Anfinsen et al., 1961). There have been many attempts to understand how. The two main avenues have been (1) the direct use of energy-minimization techniques (Momany et al., 1975; Levitt, 1976; Robson & Osguthorpe, 1979) and (2) a two-step process that converts the sequence into a secondary-structure representation followed by the construction of a three-dimensional structure by the packing together of the secondary elements (Richmond & Richards, 1978; Rose, 1979; Cohen et al., 1979, 1980, 1981a,b, 1982; Sternberg et al., 1982; Cohen & Sternberg, 1980; Sternberg & Cohen, 1982).

Energy minimization is based on sound chemical principles, but it has not been particularly successful for protein structure. The difficulties arise from the vastness of the conformation space, the mathematical limits of the optimization algorithms, and the inadequacies of the existing potential functions to

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0006-2960/83/0422-4894$01.50/0