Staphylococcal Nuclease and Pancreatic DNase Cleave the DNA within the Chromatin Core Particle at Different Sites*

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Staphylococcal nuclease (micrococcal nuclease) and pancreatic DNase (DNase I) were used to digest HeLa chromatin core particles which had been labeled with $^{32}$P at their 5'-DNA termini. In contrast to DNase I, which cleaves core particle DNA at 10-nucleotide intervals from the 5' termini, staphylococcal nuclease cleaves core particle DNA at different sites, both fewer in number and less regularly spaced. Thus, it is unlikely that simple physical protection of DNA is the sole mechanism whereby chromosomal proteins restrict the nucleolytic cleavage of chromatin; furthermore, it seems likely that these nucleases may recognize different geometric configurations along the chromatin core particle.

The purpose of the present study was to determine whether two nucleases, staphylococcal nuclease (EC 3.1.4.7) and pancreatic DNase (EC 3.1.4.5), each cleave a given nucleoprotein molecule (the chromatin core particle) at identical sites. Both nucleases have been used extensively to study chromatin structure and function, and they exhibit certain similarities in their digestion of the DNA in chromatin. For example, both enzymes digest chromatin DNA more slowly than protein-free DNA, and both cleave chromatin at specific, nuclease-sensitive regions, rather than at random (1-8). DNase I digestion generates a series of DNA fragments which, under denaturing conditions, contain multiples of 10 nucleotides (6); digestion of chromatin by staphylococcal nuclease generates a series of double-stranded DNA fragments which, under denaturing conditions, initially are "indistinguishable" from those produced by DNase I and are "separated by 10 base pairs in length" (9, 10). However, previous studies of the products of nuclease digestions utilized stained polyacrylamide gels of the DNA fragments; such analyses reveal the intervals at which nuclease-susceptible sites are spaced, but do not yield information as to the precise location of any site. There are many hypothetical distributions of nuclease-susceptible sites in chromatin which, upon nuclease digestion, will generate DNA fragments containing multiples of 10 nucleotides (or 10 base pairs). For example, consider the chromatin core particle, which contains a 140 base pair segment of DNA in a nucleoprotein complex. Following DNase I digestion and electrophoretic analysis of the DNA products, stained polyacrylamide gels reveal 14 bands, containing DNA fragments which are multiples of 10 nucleotides in length. Such a pattern could, but does not necessarily, reflect the presence of DNase I-susceptible sites at each 10-nucleotide interval along core particle DNA; the identical distribution of fragment sizes would also result if DNase I cleaved only at sites 10, 20, 30, 70, and 100 nucleotides from the end of core particle DNA. There are many additional distributions of cleavage sites which would generate similar DNA fragment patterns. Thus, the observation that during the digestion of chromatin, two nucleases generate similar patterns of stained DNA fragments cannot be taken as evidence that the two enzymes cleave the nucleoprotein at the same sites.

Furthermore, other types of evidence suggest that staphylococcal nuclease and DNase I do not cleave chromatin in identical fashion. First, staphylococcal nuclease preferentially cleaves the DNA between adjacent chromatin subunits, while initially leaving intact the DNA associated with the core particle (5, 11-13); in contrast, DNase I shows no preference for these bridge regions (6). Second, DNase I preferentially digests the transcribed sequences in chromatin DNA, while staphylococcal nuclease shows no such preference (14-16). These observations suggest that the two nucleases may not recognize the same sites in chromatin DNA.

Since nucleases have been so widely used to study chromatin structure and since the factors which influence their interaction with chromatin are not well understood, it is important to know whether each enzyme cleaves the nucleoprotein at the identical sites, or, as some of the evidence suggests, whether one nuclease cleaves chromatin at sites different from those cleaved by the other. We have previously described a method for localizing nuclease-susceptible sites within the chromatin core particle. Briefly, the technique involves labeling with $^{32}$P the 5'-DNA termini of the nucleoprotein; following nuclease digestion, the DNA fragments are analyzed by polyacrylamide gel electrophoresis and autoradiography. The distribution of bands on the autoradiogram reflects the distances from the 5'-end at which nucleolytic cleavage occurred; the intensity of a given band reflects the relative susceptibility of that site to cleavage (17). We have used this technique to compare the distribution of staphylococcal nuclease-susceptible sites along core particle...
DNA to the distribution of DNase I-susceptible sites. The results show that the cleavage sites for the two enzymes differ. These findings imply that the two nucleases may recognize different structures within chromatin, even though the size distribution of the DNA fragments they generate is similar. Furthermore, the results suggest that the direct, physical protection of DNA is not the only mechanism whereby chromosomal proteins may restrict the nucleolytic cleavage of chromatin.

**MATERIALS AND METHODS**

Staphylococcal nuclease (NFCP) and DNase I (DPFF) were from Worthington Biochemical Corp. Cell culture, preparation of nuclei, preparation of nucleosomes, labeling of nucleosomes using polynucleotide kinase and [γ-32P]ATP, and analysis of DNA fragments by polyacrylamide gel electrophoresis and autoradiography were as previously described (13, 17-21).

Digestions were performed at a nucleosome concentration of 10 A₁₀₀ units/ml at 37°C in either 0.5 mM CaCl₂, 0.5 mM MgCl₂, 5 mM Tris/Cl, pH 8, or 1 mM CaCl₂, 5 mM Tris/Cl, pH 8. The presence of calcium ion increases DNase I activity, in agreement with the observations of Price, who used protein-free DNA as a substrate (22). Neither enzyme contains detectable protease activity, as measured by discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis of core particle proteins following digestion.

**RESULTS**

Both the photograph (Fig. 1) and densitometric scans (Fig. 2) of stained gels show that the pattern of DNA fragments generated during staphylococcal nuclease digestion of core particles differs from the pattern generated during digestion by DNase I. The DNase I digests show the typical pattern of bands containing DNA fragments which are multiples of 10 bases in length; the scan has well defined peaks and a low background. In contrast, the DNA fragments generated during staphylococcal nuclease digestion do not necessarily contain multiples of 10 bases; furthermore, the peaks are not as well defined, and there is a relatively high background. DNA fragment distributions with similar features have been obtained previously by others (8, 9, 11, 12, 23). The relatively high background presumably reflects an increased heterogene-

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**Fig. 1.** Stained polyacrylamide gel (12% acrylamide, 7 M urea) of DNA fragments generated during the digestion of core particles by staphylococcal nuclease or DNase I. A to D, digestion with staphylococcal nuclease (400 units/ml) in 1 mM CaCl₂, 5 mM Tris/Cl, pH 8, for: A, 0 min; B, 4 min; C, 8 min; D, 16 min. E to H, digestion with DNase I (60 units/ml) in 10 mM MgCl₂, 5 mM Tris/Cl, pH 8, for: E, 0 min; F, 1 min; G, 2 min; H, 4 min. I to K, digestion with DNase I (50 units/ml) in 1 mM CaCl₂, 5 mM Tris/Cl, pH 8, for: I, 1 min; J, 2 min; K, 4 min.

**Fig. 2.** Densitometric scans of stained polyacrylamide gels containing DNA fragments generated during the digestion of core particles by staphylococcal nuclease or DNase I. Upper, digestion with staphylococcal nuclease (400 units/ml) in 1 mM CaCl₂, 5 mM Tris/Cl, pH 8, for 16 min. Middle, digestion with staphylococcal nuclease (same conditions as above) for 16 min. Lower, digestion with DNase I (50 units/ml) in same buffer as above for 1 min. **Ordinate** is linear with optical density.
neity in the DNA fragment lengths, perhaps caused by an exonuclease activity of the staphylococcal enzyme. The overall pattern of DNA fragments generated by staphylococcal nuclease remains rather constant during the course of digestion, except for the disappearance of the starting material (the peak at 140 nucleotides), in agreement with previous findings (8). In general, these results suggest that the distribution of staphylococcal nuclease-sensitive sites along core particle DNA is different from the distribution of DNase I-sensitive sites.

In order to determine the location of nuclease-susceptible sites, HeLa core particles are labeled at the 5'-DNA termini using polynucleotide kinase and [γ-32P]ATP. Following nuclease digestion of such labeled particles, the purified DNA fragments are analyzed by polyacrylamide gel electrophoresis and autoradiography. Previous results demonstrated that the HeLa core particle contains a DNase I-sensitive site at each 10-nucleotide interval from the 5' termini and that these sites differ in their susceptibility to cleavage by the enzyme (17, 24).

The autoradiogram shown in Fig. 3 demonstrates that the staphylococcal nuclease-sensitive sites are not located at 10-base pair intervals along core particle DNA. In contrast to DNase I, the staphylococcal enzyme does not cleave the core particle at regular intervals; the predominant bands on the autoradiogram contain approximately 115, 102, 86, 53, 32, and 21 nucleotides; the less intense bands contain approximately 93, 75, 66, and 41 nucleotides. The DNA fragment lengths in the appropriate pairs of bands add up to approximately 140 nucleotides (for example, 115 + 21, 102 + 32, 86 + 53); this would be expected for an enzyme which cuts both DNA strands at the same locus. In addition, these cleavage sites are spaced at intervals of (roughly) 10 base pairs. Thus, a stained gel of the products generated during staphylococcal nuclease digestion of nuclei or chromatin could show bands of DNA fragments separated by approximately 10 base pairs (9).

A scan of a typical autoradiogram (Fig. 4) demonstrates that the central segment of core particle DNA, 60 to 80 nucleotides from the 5' termini, is relatively resistant to digestion by staphylococcal nuclease; this same region is also relatively resistant to cleavage by DNase I and an endonuclease from Aspergillus oryzae (24). This finding is consistent with the previous suggestion that in this region, the nuclease cannot form the correct complex with divalent cation and DNA required for catalysis. The remaining DNA segments are readily cleaved by staphylococcal nuclease. A scan for only a single extent of digestion is shown, since the pattern remains about the same (except for the peak at 140 nucleotides) during a course of digestion from about 10% acid-soluble DNA to 60% acid-soluble.

DISCUSSION

The present experiments demonstrate that, during the digestion of core particles, staphylococcal nuclease and DNase I generate different patterns of fragments containing the 5'-DNA termini. The reason(s) for this is unknown. For example, it is conceivable that staphylococcal nuclease might make its sites...
initial cuts at sites identical with those cleaved by DNase I; then, due to an inherent feature of the staphylococcal enzyme's cleavage mechanism, the initial DNA fragments are shortened by varying amounts. However, if this were the case, it would be difficult to explain the observations that (a) on the autoradiogram, the lengths of DNA in appropriate pairs of bands add up to 135 to 140 base pairs and (b) there are apparently fewer staphylococcal nuclease-sensitive sites than DNase I-sensitive sites. Thus, although this possibility cannot be ruled out, it seems unlikely; if therefore seems reasonable to assume that the distribution of bands on the autoradiogram does, in fact, reflect the actual distribution of staphylococcal nuclease-susceptible sites along core particle DNA and that these sites are indeed different from those susceptible to DNase I.

The identification of sites susceptible to cleavage by staphylococcal nuclease and DNase I is relevant to the question of how chromosomal proteins restrict the nucleolytic cleavage of chromatin DNA. Thus, chromatin proteins might affect the digestion (a) directly by physically protecting certain DNA segments from enzymatic attack or (b) more indirectly by affecting the configuration of segments of DNA without necessarily covering them physically. In the first instance, both staphylococcal nuclease and DNase I would presumably attack identical sites in a nucleoprotein, namely, those DNA segments not directly protected by proteins. In the second case, the enzymes might cleave at different sites, due to their recognition of different DNA configurations within chromatin. The observations made here, that the two nucleases cleave the core particle at different sites, suggests that the direct, physical protection of DNA is not the only factor which determines the distribution of nuclease-sensitive sites within a nucleoprotein. Furthermore, staphylococcal nuclease (Mr = 16,800) is a smaller molecule than DNase I (Mr = 31,000); this difference might allow the staphylococcal enzyme to attack more sites than DNase I along core particle DNA, if physical protection of the DNA by chromosomal proteins was the only factor restricting its nucleolytic cleavage. The results indicate that there are apparently fewer staphylococcal nuclease-sensitive sites within the core particle, again arguing that physical protection of the DNA by protein is not the sole factor which determines the distribution of nuclease-sensitive sites along chromatin DNA. The central segment of core particle DNA, 60 to 80 nucleotides from the 5' termini, is relatively resistant to cleavage by both nucleases; this may reflect physical protection of this region by chromosomal proteins.

Staphylococcal nuclease is a basic protein, with an isoelectric point of 9.6; the isoelectric point of DNase I is 4.7. The amino acid composition of a nuclease could conceivably affect the interaction between the enzyme and the nucleoprotein, leading to differences in the sites for nucleolytic cleavage. However, both DNase II (isoelectric point 10.2) and the Aspergillus DNase (isoelectric point 9.2) cleave the core particle at the same sites as does DNase I (24). Thus, the isoelectric point of a nuclease is not a primary factor in determining the sites for enzymatic attack.

Our previous experiments demonstrated that certain nucleases do cleave the core particle at the identical sites (24). One property that these enzymes (DNase I, DNase II, and the A. oryzae endonuclease) have in common is the preferential hydrolysis of double-stranded DNA. Thus, a major difference between these enzymes and the staphylococcal enzyme is that staphylococcal nuclease preferentially hydrolyzes single-stranded nucleic acids (for review see Refs. 25 to 27). This difference in substrate preference implies that important differences exist between staphylococcal nuclease and DNase I, both in the geometry of their active sites and in the DNA conformation which they recognize. It seems quite possible that the reason staphylococcal nuclease and DNase I cleave the core particle at different sites is because they recognize and cleave regions of chromatin which have different configurations. However, the details of such hypothetical differences in nucleoprotein configuration are unknown.

The results of these and previous experiments suggest that the three-dimensional geometry of the nucleoprotein substrate may be an important factor in the selection of cleavage sites by nucleases. This may be an example of a more general phenomenon, namely, that other proteins which interact with chromatin may also recognize specific configurations of the nucleoprotein. Alterations in protein-DNA interactions within chromatin (produced, for example, by chemical modification of the histones or by the interaction of chromatin with nonhistone proteins) could lead to changes in the shape of the nucleoprotein without substantial alteration in the amount of DNA which is physically protected by protein. Such changes in configuration could alter the interaction between chromatin and other proteins and may constitute the basis for the observation that DNase I preferentially cleaves the transcribed sequences in chromatin DNA (14, 16). Thus, small changes in chromatin structure may play important roles in the specific expression of genetic information.

Acknowledgments—I thank Dr. Robert T. Simpson for his guidance, encouragement, and constructive criticism. I thank Drs. Arnold Stein and Minou Bina-Stein for reviewing the manuscript and Ms. Linda Probst for photographic assistance.

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