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DNase II digestion of the nucleosome core: precise locations and relative exposures of sites

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ABSTRACT

The precise locations and relative exposures of the DNase II-accessible sites in the nucleosome core DNA are determined using techniques previously employed for the enzyme DNase I. It is found that there are a number of similarities between the site exposure patterns for the two enzymes but that in general the DNase II seems to discriminate less among adjacent sites' accessibilities than does DNase I. The two enzymes attack essentially the same positions in the DNA, the average difference between the precise location of a site being less than one-half base for the two enzymes. Such close similarities in the digestion patterns of two enzymes with such different mechanisms of scission show that the patterns reflect the structure of the nucleosome core and not merely the properties of the particular enzyme used.

INTRODUCTION

Nucleases have been extensively used as structural probes to gain detailed information about the organization of DNA in the nucleosome core (for reviews see references 1-4). DNase I has been used in particular in nucleosome digestion studies, and both the relative exposure of DNA sites (5, 6) as well as the precise location of those sites (7) have been determined. These results have been used in combination with x-ray crystallographic data to describe a model for the structure of the nucleosome core (8).

However, in using a structural probe as large and as complicated as a nuclease, it is necessary to attempt to assess how much of the result stems from the nucleosome structure and how much is characteristic of the particular nuclease used. To try to determine the contribution of the nucleosome structure to the data, several nucleases with different mechanisms of scission can be employed to digest nucleosomes. Common features in the digestion results of several nucleases will most likely reflect nucleosome structure rather than individual cutting preferences of a

particular nuclease.

This paper describes the quantitative determination of site exposure as well as the precise site locations for the digestion of nucleosome cores by DNase II, a nuclease with cutting characteristics significantly different from DNase I. The techniques employed are the same as those used in similar determinations with DNase I (7), and the results for the two enzymes are compared.

MATERIALS AND METHODS

Nucleosome cores were prepared from beef kidney and labelled with ^{32}P at their 5' termini essentially as described (5, 6, 7). Kinase labelling was carried out in an incubation mix of 25 µl which contained 230 pmoles nucleosome cores, 750 µmoles ^{32}P ATP (133 Ci/mmole, Amersham), 10 mM $MgCl_2$, 4mM potassium cacodylate, 0.2 mM EDTA, 50 mM Tris Cl (pH8.0), and 25 units polynucleotide kinase (Boehringer). After 45 minutes incubation at 37°C, 25 µl of 11 mM EDTA plus 260 mM unlabelled ATP was added and the mix put on ice. Nucleosome cores were digested immediately with nuclease in an incubation mixture of 25 µl containing 4 µl of the above labelled core preparation plus 0.8 A^{260} units unlabelled nucleosome cores, 28 mM potassium cacodylate (pH 6.0), 0.1 mM EDTA, nuclease [50 units DNaseII (Sigma) or 1.25 units DNase I (Sigma)], with 5mM $MgCl_2$ or 1 mM EDTA, depending on the experiment. Incubation was at 37°C for 20, 40, 60, 120, and 300 seconds, after which a 5 µl aliquot was added to 5 µl 10mM EDTA, 300 mM Tris-Cl (pH8.8) on ice. The samples were then processed and electrophoresed in high-MBA (methylene-bis-acrylamide) polyacrylamide gels of various acrylamide percentages. Such gels are capable of resolving unique chain-length fragments of mixed sequence DNA well into the range of 160 bases (see reference 7 for complete description). Data from multiple time points and from several gels were used to determine rate constants of nuclease attack at individual nucleosome core DNA sites (5, 6) as well as the precise location of those sites with respect to the 5' end of the DNA (7).

Fragment lengths were assigned to individual bands in both DNase I- and DNase II-produced patterns (see Figure 1) by electrophoresis in adjacent channels samples of end-labelled DNA from a DNase I digest of nuclei, a pattern whose bands have been assigned fragment lengths by coelectrophoresis with multiple sequenced restriction fragments (7,10). The unknown channel was then sized by matching bands at a position low enough in the gel where the lineup between channels was obvious (see also discussion relevant to

Figure 3 below). Crosschecks were also made among gels for assignments. Of course once one band in a channel is assigned a fragment size, the sizes of all the other bands in the channel can be determined by counting from that band.

Unique chain length DNA fragments containing mixed sequence and ^{32}P-labelled 5' termini (See Figure 3) were obtained as follows: Rat liver nuclei were digested with DNase I (6). The DNA was extracted, treated with bacterial alkaline phosphatase, and labelled at the 5' ends with γ-^{32}P-ATP and T4-polynucleotide kinase (6). The labelled DNA was then fractionated by electrophoresis in a high-MBA polyacrylamide gel (20% acrylamide, 2.4% methylene-bis-acrylamide, see reference 7). In such a gel mixed sequence DNA fragments of a unique chain length migrate as a single band, and the band corresponding to a fragment 13 bases in length was cut out of the gel and eluted into 1M triethylammonium bicarbonate (pH 8.0) by shaking overnight at 37°C. The eluate was dialyzed against water, after which it was concentrated by evaporation using a Savant Speed Vac concentrator. An aliquot of the concentrate was then subjected to the guanine-specific reaction as described by Maxam and Gilbert (9). A sample was then electrophoresed in a 20% acrylamide, 2.4% MBA polyacrylamide gel along side samples of 5' end-labelled DNA from a DNase I digest on nuclei. Bands were assigned fragment sizes by counting down from characteristic bands in the gel, specifically the band corresponding to the fragment 20 bases long in band 'B2' (see reference 10).

RESULTS

Both the precise location of sites as well as the relative exposure of the sites of DNase II attack on the DNA of the nucleosome core were determined. Since DNase II has been reported to digest nucleosome cores differently in the presence and absence of divalent cations (11, 12), DNase II digestions were carried out with 1mM EDTA or with 5mM $MgCl_2$ present.

Relative Exposure of Sites: The pattern of relative exposure of each of the nucleosome DNA sites was determined for DNAse II + EDTA and DNase II + $MgCl_2$ employing the procedure previously used for DNase I (5, 6). Originally used on the nucleosome core, this procedure came to be called 'footprinting'(13) when it was subsequently used to locate proteins bound to specific DNA sequences (this term emphasizes the pattern of protection

of the DNA by the protein). Nucleosome cores were labelled at the 5' termini of their DNA and digested for varying amounts of time with either DNase I, DNase II + EDTA or DNAse II + $MgCl_2$. Each sample was deproteinized and electrophoresed in a high-MBA polyacrylamide gel, after which the gel was autoradiographed (See reference 6 for general methods).

Figure 1 shows an autoradiograph of a gel upon which were electrophoresed DNA samples from two minute digestions of nucleosome cores employing DNase II + EDTA (Fig. 1A), DNase II + $MgCl_2$ (Fig. 1B) and DNase I (Fig. 1C). Figure 1D shows a densitometer tracing of the autoradiograph from a five minute digestion with DNase II + EDTA. Each of the broad bands on the gel corresponds to a cutting site on a strand of the nucleosome core DNA, with the corresponding fragment size of the band indicating the distance in bases of the site from the 5' end of the core DNA. The amount of radioactivity in each band can be used to determine the apparent first order rate constant $\underline{k}$ for enzyme attack at the corresponding site (5, 6). These constants are listed in Table 1 and plotted as a bar graph in Figure 2.

It can be seen that DNase II cuts at all sites (Figure 2A), with the ends of the DNA being attacked somewhat more readily than the central portion. The addition of $MgCl_2$ to the DNase II digestion (Figure 2B) has little qualitative effect on the cutting pattern: there is an overall decrease of about one-third in the amount of cutting but there are no striking changes among the relative exposures of the sites.

A comparison of the DNase II exposure pattern (either with (Figure 2B) or without (Figure 2A) $MgCl_2$) with that of DNase I (Figure 2C) shows both similarities and differences. Both patterns exhibit a strongly masked site S11 flanked by two more heavily cut sites, S10 and S12. However, the DNase II pattern in general tends to be more uniform than that of DNase I, especially toward the middle of the DNA. Thus while site S3 is also cut relatively infrequently by both enzymes, DNase I cuts the neighboring sites S2 and S4 in a contrastingly heavy manner, but DNase II does not. This higher 'contrast' between neighboring sites can be said to be generally true for the DNase I pattern (sites S3, S6, S8, and S11 are each infrequently cut sites flanked by more heavily cut sites) but, with the exception of S11, is not characteristic of the DNase II pattern. Put another way, DNase II does not seem to differentiate among sites' accessibilities as effectively as does DNase I. We discuss this further below.

Location of sites:

a.) Electrophoretic mobility effect of a terminal phosphate: As

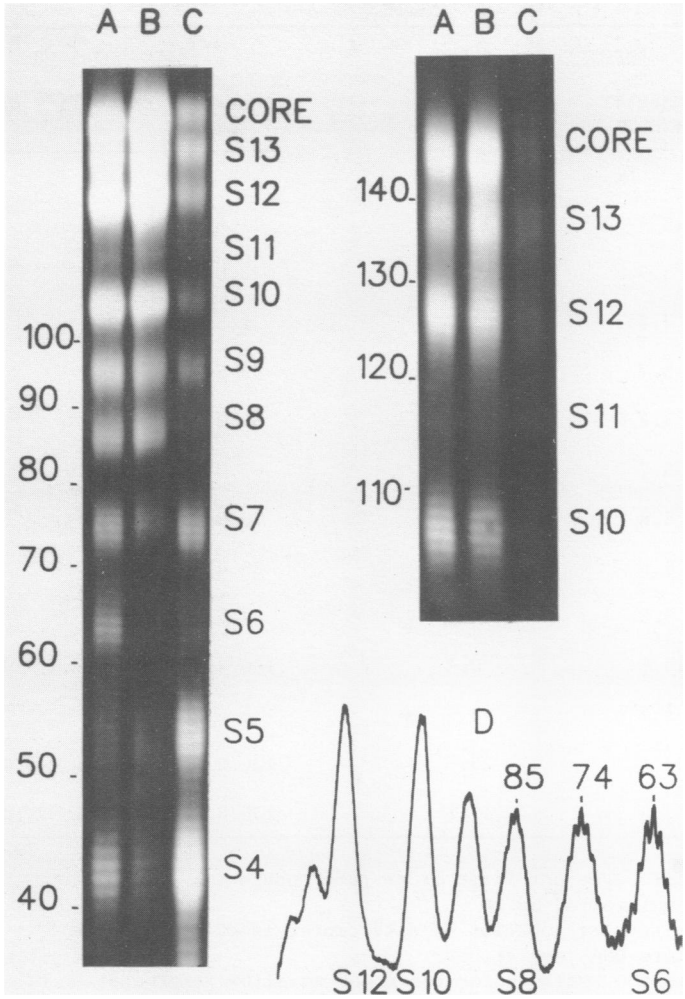

Figure 1: DNase I and DNase II digests of end-labelled nucleosome cores. ^{32}P-end-labelled nucleosome core were digested with nuclease, after which the DNA from the digest was electrophoresed in a high-MBA (6% acrylamide, 1% MBA) polyacrylamide gel. An autoradiograph of the gel is shown, with the left and right autoradiographs coming from channels which were subjected to short and long electrophoresis times, respectively. To the left of each autoradiograph is indicated the fragment length in bases, while to the right is indicated the corresponding site number. Digestion was for 2 minutes by A.)DNase II + EDTA, B.)DNase II + $MgCl_2$, C.)DNase I + $MgCl_2$. Panel D.) shows a microdensitometer tracing of the autoradiograph of a similar gel from a 5 minute digestion by DNase II +EDTA. Fragment lengths in bases are listed in above the trace, as determined by comparison to end-labelled DNA from a DNase I digest of nuclei (7). Sites corresponding to the various bands are listed below the trace.

Table 1

Site	Exposure [a]		Site Location [b]		
	DNase II + EDTA	DNase II + Mg	DNase II + EDTA	DNase II + Mg	DNase I + Mg
S1	15.4	11.7	11.6	13.1	12.5
S2	3.9	1.9	23.0	c	22.6
S3	2.4	1.7	32.2	32.4	c
S4	5.7	2.3	42.5	42.9	42.4
S5	3.2	2.0	53.2	53.0	53.1
S6	5.7	1.4	63.2	63.6	63.0[d]
S7	6.8	2.6	73.9	74.6	73.8
S8	6.5	3.7	85.4	85.4	c
S9	9.3	4.5	94.5	94.6	94.5
S10	15.6	9.3	106.4	106.4	106.1
S11	3.4	3.2	c	c	c
S12	34.6	23.5	126.8	127.0	126.2
S13	35.3	37.1	136.8	136.8	136.1

a. Table lists apparent first order rate constant k in reciprocal seconds multiplied by 10^4.
b. Values given are location of site center in bases from the 5' end of core DNA (see 7).
c. Peak site too small and/or too broad to allow determination of center.
d. S6 showed a small peak of cutting here which allowed tentative site location.

had been determined previously for DNase I (7), the precise location of the nucleosome core sites attacked by DNase II have been mapped with respect to the 5' terminus. Briefly, this involves a DNase II digestion of nucleosome cores whose 5' termini have been labelled with ^{32}P. The DNA is then extracted and electrophoresed in high-MBA polyacrylamide gels of various acrylamide concentrations.

However, in order to directly compare the location of the sites

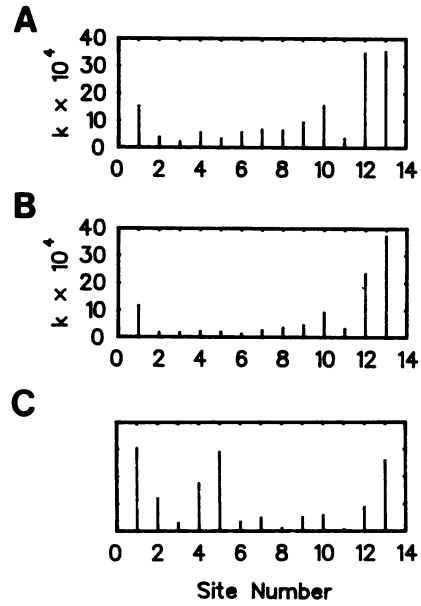

Figure 2 Exposure of nucleosomal sites to nuclease attack.
Data is taken from Table 1. The rate constant for attack (k) at each site is plotted for digestion with DNase II + EDTA (A) and DNase II + $MgCl_2$ (B). The bottom graph (C) is a plot of the data for DNase I from reference 6. For ease of comparison this DNase I plot has been normalized to the DNase + EDTA plot by making the sums of bar heights for each enzyme equivalent.

attacked by DNase I with those attacked by DNase II, it is necessary to assign absolute fragment lengths to the bands in the DNase II-produced pattern. Such an assignment was made for the DNase I-produced bands by coelectrophoresis with sequenced DNA fragments (7, 10), so the DNase II-produced bands could now in turn be assigned sizes by coelectrophoresis with such a 'standardized' DNase I-produced pattern. However, DNase II produces a fragment with a 3' terminal phosphate (14) while DNase I produces a fragment with a 3' terminal hydroxyl (15)(both contain 5', ^{32}P-labelled phosphates).

This extra phosphate on a DNase II-produced fragment would be expected to result in a higher mobility for such a fragment when compared to a DNase I fragment of the same length, raising the possibility that the DNase II fragment would be assigned an erroneously small length if sized against a DNase I-produced fragment. However, such an error would be unlikely if the

extra phosphate increased the mobility of the fragment by less than one half base, because if this were the case, one would be unlikely to assign a size one full base 'out of step'. Thus in order to unambiguously assign fragment lengths to the bands in a DNase II-produced pattern in a gel, one needs to know the effect that the extra phosphate has on the electrophoretic mobility of a DNase II-produced fragments; specifically, is it less than or greater than the equivalent of about half a base.

This effect of a terminal phosphate was determined as follows: the DNA fragments from a DNase I digest of nuclei were labelled at their 5' ends with ^{32}P and fractionated by electrophoresis on a high-MBA polyacrylamide gel (See Materials and Methods). The band corresponding to a fragment 13 bases in length was cut out of the gel and the fragment eluted, giving a fragment containing 13 bases of mixed sequence, a 5' ^{32}P phosphate and a 3' hydroxyl. This fragment was then partially degraded using the guanine-specific DNA sequencing reaction of Maxam and Gilbert (9), a degradation which yields a continuous series of smaller fragments, all of which have 3' phosphate termini. This digest was then electrophoresed in a high-MBA polyacrylamide gel in a channel adjacent to the original 5' end-labelled DNA from a DNase I digest of nuclei, i.e. a continuous series of fragments which contain 3' hydroxyl termini.

Figure 3 shows an autoradiograph of the gel. Here it can be seen (Figure 3B) that the degradation has produced a series of lower bands, one of which corresponds to a fragment 12 bases long (resulting from removal of one nucleoside from the 3' end of the original 13 base fragment), although this band is partially obscured by the large amount of radioactivity in the band corresponding to the original 13 base fragment. The presence of this 12-base band indicates that the 3' terminal phosphate does not grossly alter the mobility of the fragment, i.e. it does not increase the mobility by more than the equivalent of one base. Furthermore, when one compares bands further down in the channels, it can be seen that the presence of the 3' terminal phosphate (Figure 3B) causes the fragment to move the equivalent of only 0.1-0.2 base faster than the same length fragment containing a 3' hydroxyl (Figure 3A)(see also reference 7). Such a slight increase (<0.5 base) in the electrophoretic mobility of a fragment resulting from the addition of a single terminal phosphate has also been observed in a number of studies involving unique sequence DNA fragments electrophoresed in polyacrylamide gels containing lower levels of MBA (16, 17).

This minimal effect (0.1-0.2 base) of the additional phosphate on the

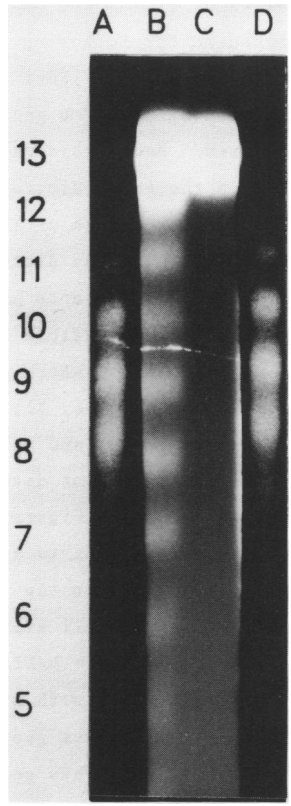

Figure 3 Terminal phosphate effect on the electrophoretic mobility of a fragment.

A population of 13 base mixed sequence fragments labelled at their 5' termini were degraded using the guanine-specific reaction of Maxam and Gilbert (9). Following the degradation reaction an aliquot was electrophoresed in a high-MBA polyacrylamide gel, after which the gel was autoradiographed. Shown above is the autoradiograph of a portion of the gel to which was applied: A.) and D.)5'-end labelled DNA from a DNase I digest of nuclei. B.) 13-base fragment after degradation. C.) 13-base fragment before and after degradation. Numbers at left indicate fragment length in bases. Due to different amounts of sample applied, channels A and D had significantly more radioactivity in this region than had channels B and C, so to allow direct comparison of band positions channels A and D were exposed longer during printing of the photograph.

mobility of a DNase II-produced fragment means that the DNase II-produced pattern can be sized directly from the known fragment lengths in the DNase I-produced pattern without fear of making a mistake of one base or more, i.e. going one base 'out of step'. This in turn permits a direct comparison of

the locations of nucleosome sites of attack for DNase I and DNase II, which have been determined as described in the following section.

b.) Determination of site locations: Figure 1D shows a representative densitometer tracing of the autoradiograph of a gel channel upon which a DNase II sample was electrophoresed. Each broad band (i.e. the envelope of the distribution of fine bands) in the autoradiograph corresponds to a site which is cut by the nuclease, while the length of the DNA fragment corresponding to center of the envelope of the distribution is the distance in bases of that site from the 5' end of the core DNA. Site locations for the three digestion conditions are listed in Table I, and the results are plotted in Figure 4 as excess over 10.0 x n, where n is the site number starting from the 5' end of the core DNA (5, 6, 7).

As found previously for both native (7) and poly-dAT reconstituted (18) nucleosome cores and as shown in an independent determination here (Table 1, Figure 4A), this DNase I site location plot (Figure 4A) shows a relatively steep section in the center, which corresponds to a periodicity of about 10.6 (sites S4-S10), comparatively flat sections on the two ends which correspond to a periodicity of about 10.0, with an overall average value of about 10.4. The DNase II + EDTA (Figure 4B) and DNase II + $MgCl_2$ (Figure 4C) patterns both show a form similar to that of DNase I, with a steep section in the center (periodicity 10.6 - 10.9) and a flatter section toward the ends, especially the 3' end (periodicity 10.0). This general similarity supports the notion that the form of such a curve reflects nucleosome structure and not merely the particular preference of the nuclease used. Both DNase II patterns show a marked discontinuity at S8-S9. This effect is most likely due to the fact that histones considerably alter nuclease access to site S8, especially when considered with the fact that S8 is hardly cut at all by DNase I (5, 6).

The precise locations of the sites attacked by DNase I are compared directly with those attacked by DNase II in Figure 4D. Here it can be seen that at a given site the two enzymes cut the sugar-phosphate backbone at essentially the same location: the centers of the site locations for the two enzymes are on the average less than one-half base from one another. Thus not only are the spacings between individual sites very similar for the two enzymes, but the actual site locations are very similar as well. This latter observation is not consistent with the proposal of Sollner-Webb et.al. (12) in which DNase I cut is thought to cut nucleosome DNA at a location displaced one base in the 5' direction from the location which is cut by

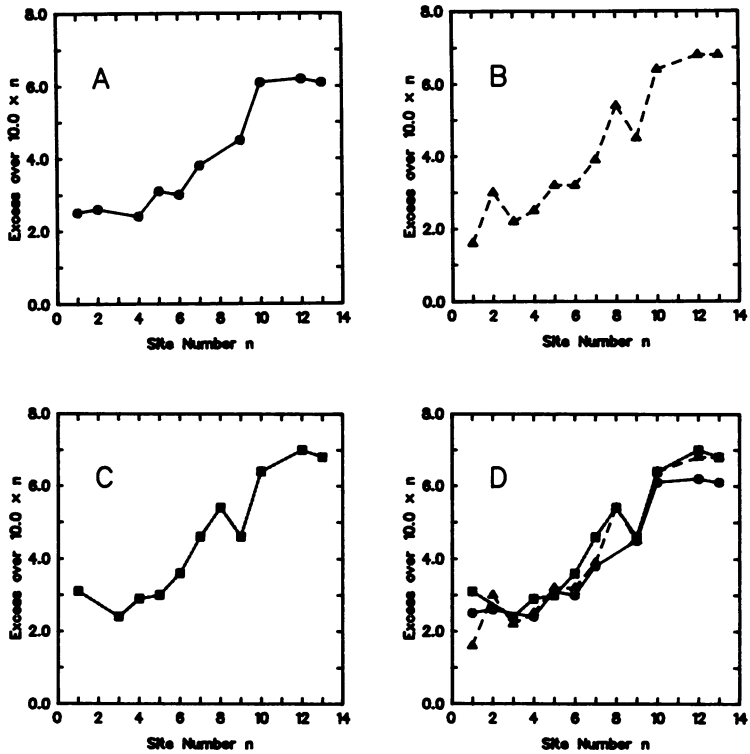

Figure 4: Graphs of the locations of sites of attack for DNase I and DNase II.

Data is taken from Table I. Plotted are the precise locations of sites of attack in nucleosome core DNA for: A.) DNase I + $MgCl_2$; B.) DNase II + EDTA; C.) DNase II + $MgCl_2$; D.) all three sets of data plotted on the same graph.

DNase II (see discussion below).

DISCUSSION

In order to distinguish those features which are characteristic of nucleosome core structure from those which are specific for the particular nuclease employed, we have determined the precise location and relative exposure of sites of attack on the nucleosome core for two enzymes, DNase I and DNase II. Common features in the digestion characteristics of these two enzymes are likely to be representative of nucleosome core structure.

Relative exposure of sites: The site exposure patterns for DNase I and DNase II have some features in common and some differences. For both

enzymes the site S11 is masked to nuclease attack when compared to the adjacent sites, S10 and S12. Figure 2C shows the region centered about site S3 is also attacked by DNase I in a very similar manner to the region around S11 (i.e. S3 is masked relative to beighboring sites, S2 and S4) and this similarity has been interpreted by us (5, 6, 8) to result from common protection by protein of two superhelical turns of DNA in a nucleosome model having 80 base pairs per superhelical turn.

If the sites S3 and S11 are both points of intimate association of DNA with protein as indicated by these DNase I results, then one might expect site S3 to be protected from DNase II attack as well. Figure 2A and 2B show that this is in fact the case, at least with respect to the overall cutting of the DNA: site S3 is cut by DNase II less frequently than just about any other site in the nucleosome core DNA. However, unlike the DNase I pattern, site S3 does not stand out as a relatively infrequently cut site because its neighboring sites, S2 and S4, are also cut at a comparable level by DNase II. Thus the general picture of the results of the two enzymes is that there are some similarities between the site exposure patterns of DNase I and DNase II, and where there are differences, DNase I seems to produce more contrast between neighboring sites than does DNase II. It may well be that DNase II, being larger (38,000 daltons) than DNase I (31,000 daltons), is less able to gain differential access to various sites. Furthermore, not only may the two enzymes have different sizes and shapes, but it is likely that the particular configurations of the binding and cutting sites on the two enzymes differ.

Site Locations: Although there are some differences between the site exposure patterns for the two enzymes (primarily due to the rather uniform suppression of DNase II cutting in the middle of DNA), the patterns of site locations (Table I, Figure 4) are quite similar. Both patterns have quite a similar form, with a steep central section (Figure 4) corresponding to a spacing of about 10.7 bases between sites, relatively flat sections toward the termini of the DNA corresponding to a spacing of about 10.0 bases between sites,and an overall average spacing of about 10.4 bases between sites.

In addition, not only are the spacings between sites for the two enzymes very similar, the sites themselves are in essentially the same absolute location. That this is the case derives from the fact that absolute sizes can be readily assigned to the bands in a DNase II-produced pattern (Figure 1)(just as has been done for the DNase I-produced pattern

(7)) when one knows (from Figure 3) that the presence of a phosphate on the 3' termini of the DNase II-produced fragments has a minimal effect on the electrophoretic mobility of those fragments when compared to the analogous DNase I-produced fragments. When the site locations are then compared (Table 1, Figure 4D) it can be seen that the DNase II sites are less than a half base on the average from the DNase I sites. These similarities explain our observation that the fragment size distributions for digestion of chromatin by DNase I and DNase II are also very similar (10) and that the fragment size distribution in a DNase I digest of chromatin can be fitted quite well by a calculated pattern employing rate constants and precise locations of DNase I attack on the core (7).

Using data from experiments of a design similar to those presented here as well as from experiments which involve 'filling in' of double stranded fragments with DNA Polymerase II to determine cutting stagger, Sollner-Webb et.al. (12) have proposed that DNase II and DNase I both recognize the same double-stranded site, but DNase II makes a cut one base 5' to the cut made by DNase I. The findings presented here do not agree with such a proposal since the data in Table 1 and Figure 4 show that DNase II, either with or without $MgCl_2$ present, cuts the DNA at a given site less than one-half base on the average from the point where DNase I cuts.

Furthermore, the proposal by Sollner-Webb et.al. (12) that DNase II cuts chromatin DNA to give cuts on opposite strands which are staggered by 4 base pairs (3' extending) is also not supported by our data: using the values in Table 1 in conjunction with results from the DNase I-'filling in' experiment performed in reference 7, the stagger at each of the DNase II sites can be calculated. The average of the individual stagger values calculated in this manner is 1.8 bases (3' extending) for DNase II + EDTA and 2.8 bases for DNase II + $MgCl_2$. Thus the staggers derived from the data presented here also do not agree with the 4 base stagger proposed by Sollner-Webb et.al. (12). Considering the facts that the gel system used by Sollner-Webb et.al. (12) does not resolve individual mixed-sequence DNA fragments as well as that used here and that their sizing standard was not composed of integral multiples of a single value (10), a likely explanation for the discrepancy between their results and ours is that their error limits were somewhat greater than they estimated.

Finally, the whole idea of a nuclease recognizing a double-stranded site in a nucleosome DNA (proposed by ourselves (19) for DNase I and by Sollner-Webb et.al. (12) for DNase I, DNase II and micrococcal nuclease), is

unlikely in light of the fact that the stagger is not constant but can vary from 0.7 to 3.7 bases (3' extending), depending on the pair of sites considered in Table I. This fact has been pointed out previously in a discussion of the precise locations of the DNase I cutting sites in the nucleosome core (7).

In conclusion, we find that DNase II and DNase I show similarities as well as some differences in the patterns of exposure of their sites of attack in nucleosome core DNA. A comparison of the precise locations of the sites for these two enzymes shows that they both cut the nucleosome core DNA at nearly the same phosphodiester bond, with the spacings between these sites being about 10.7 bases in the middle region of the DNA and about 10.0 bases toward the ends. However, one must be cautious in identifying these exact periodicities directly with the structural periodicity of the DNA double helix, as has been done, for example, in reference 18. We have pointed out previously (10) as well as in the accompanying paper (20) that, due to the possibility that a nuclease may exhibit different angles of attack at different sites, a distance of e.g. 10.7 bases between two given sites does not necessarily mean the screw of the DNA is 10.7 base pairs per turn between those two sites. Rather, this value is likely to be closer to 10 base pairs per turn, but a precise correlation between the structural periodicity and the spacings between sites determined here will probably need to await the results of our high resolution crystallographic studies on the nucleosome core (21, 22).

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