Micrococcal Nuclease Cleavage of Chromatin Displays Nonrandom Properties†

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ABSTRACT: A statistical analysis of the products of digestion of chicken erythrocyte chromatin by micrococcal nuclease was used to test for randomness of the cutting process. DNA fragment size classes corresponding to mononucleosome, dinucleosome, trinucleosome, tetranucleosome, and all fragments larger than tetranucleosome were evaluated. In every case, fragments in the mononucleosome and greater-than-tetranucleosome classes were produced in excess of the level expected on the basis of random cleavage while those in the dinucleosome–tetranucleosome classes exhibited a shortage. The pattern of nonrandomness appears to depend on substrate size: the *magnitude* of deviations from randomness was large when substrates of genomic size are compared with polynucleosomal segments whereas the direction of deviation is identical. Nonrandomness was independent of ionic conditions known to affect the state of chromatin condensation and also appeared to be unaffected by depletion of histones H1 and H5. The possible universality of nonrandom cleavage was suggested when other data from the literature were analyzed. Some possible mechanisms to account for this property are discussed.

The digestion of chromatin with endonucleases has been an important tool in the development of the nucleosome model of chromatin structure (Hewish & Burgoyne, 1973; Noll, 1974; Kornberg, 1974) and in subsequent studies of chromatin structure and function (Felsenfeld, 1978). It has generally been assumed that micrococcal (staphylococcal) nuclease cuts internucleosomal linker DNA at random, and, indeed, in cases where this aspect has been examined, random cutting has been observed (Burgoyne & Mobbs, 1975; Lohr et al., 1977a).

Tests for random nuclease attack have compared the observed fragmentation of chromatin with the model system of random cleavage of an infinitely long linear polymer discussed by Tanford (1961). This model gives the weight fraction (W) of an oligomer of size n as

$$W_n = np^{n-1}(1-p)^2 \qquad (1)$$

where p is the fraction of the linker regions *intact* at any given time. A plot of weight fractions for mononucleosomes through tetranucleosomes, and the class of digestion products greater in size than tetranucleosomes, as a function of percent linkers remaining intact on the basis of the random model is given in Figure 1. Using this relationship, Lohr et al. (1977a) found that the digestion of yeast chromatin by micrococcal nuclease showed no significant deviation from randomness, and Burgoyne & Mobbs (1975) reached the same conclusion regarding the digestion of rat liver chromatin by endogenous endonuclease.

However, in examining the products of micrococcal nuclease digestion of chicken erythrocyte chromatin, it appeared to us that the ratio of mononucleosomes to dinucleosomes was much higher than expected on the basis of random cleavage. We have, therefore, reinvestigated the question of randomness and developed a more rigorous test for comparing the observed products to that expected for random cleavage of an infinitely long linear polymer at regularly spaced sites (Tanford, 1961).

The studies reported here offer evidence that micrococcal nuclease cleavage of linker DNA in whole chicken erythrocyte chromatin is a nonrandom process regardless of the ionic strength (and hence the degree of chromatin condensation) in which the experiments are performed. A similar nonrandom digestion is exhibited by multimeric chromatin segments. The pattern of nonrandom cleavage is consistent: mononucleosomes are produced in excess, while the classes of small oligonucleosomes (n = 2–4) are under-represented. Possible explanations for this phenomenon are considered.

Materials and Methods

Isolation of Cells and Nuclei. Chicken erythrocytes were collected and washed in buffer consisting of 10 mM Pipes[1] and 150 mM KCl, pH 8.0, and the cells were disrupted by mixing at room temperature for 15 min in the presence of 0.4% Nonidet P-40. Nuclei were recovered after at least two washings in the same buffer by sedimentation at 375g. The amount of DNA was estimated from the absorbance at 260 nm in 1.0 M NaOH by using the relation 1.0 A_{260} = 50 μg/mL.

Digestion with Micrococcal Nuclease. Nuclease digestion buffer consisted of 10 mM Pipes and 1.0 mM $CaCl_2$, pH 8.0, which was, for some experiments, supplemented with salts to give final concentrations of 150 mM KCl, 1.0 mM $MgCl_2$, or 5.0 mM $MgCl_2$. In some experiments, NaCl and Tris buffer were used without altering the results. Nuclei were washed in digestion buffer and resuspended, digestion was initiated by adding 1 unit of micrococcal nuclease (Worthington Biochemical Corp.) per absorbance unit of chromatin DNA, and the reaction was allowed to proceed for various times at 37 °C. Enzyme activity was halted by adding Na_2EDTA to 10 mM and cooling to 0 °C.

Treatment of Digests. Phenol extraction: DNA was isolated by making the digest samples 1.0 M in NaCl and extracting according to Sollner-Webb et al. (1976) with an equal volume of chloroform–isoamyl alcohol (24:1 v/v). The phases were separated by centrifugation at 1000g for 10 min, and the aqueous phase was reextracted with an equal volume of

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[1] Abbreviations used: Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate; *Hae*III, *Haemophilus aegyptius* restriction endonuclease; CHO, Chinese hamster ovary.

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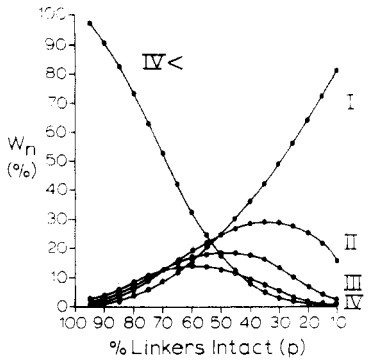

FIGURE 1: Weight fraction (W) of mononucleosomes (I), dinucleosomes (II), trinucleosomes (III), tetranucleosomes (IV), and all products greater in size than IV (IV<) expected on the basis of random cleavage as a function of percent linkers remaining intact. This profile is derived from eq 1.

phenol–chloroform–isoamyl alcohol (24:24:1 v/v). DNA was precipitated from the aqueous phase by adding 2 volumes of cold absolute ethanol and allowing to stand at −20 °C overnight. The DNA was then collected by sedimentation at 15000g for 15 min.

$NaDodSO_4$ method: Whole digest samples were precipitated by making the suspension 5.0 mM in $MgCl_2$, adding 2 volumes of cold absolute ethanol, and allowing to stand at −20 °C overnight. The precipitate was collected by centrifuging at 2000g for 15 min, and the samples were prepared for $NaDodSO_4$ gel electrophoresis by the method of Varshavsky et al. (1978).

Polyacrylamide Gel Electrophoresis. Electrophoresis was in 3.5% polyacrylamide slab gels [19:1 acrylamide/bis(acrylamide)] utilizing the buffer system of Loening (1967) and, in the case of $NaDodSO_4$-treated samples, incorporating 1.0% $NaDodSO_4$ according to Varshavsky et al. (1978). $NaDodSO_4$-treated samples were readissolved in running buffer consisting of 10% glycerol, 0.16% bromophenol blue, and 1.0% $NaDodSO_4$, heated at 55 °C for 15 min, and electrophoresed at 100 V and 75 mA for approximately 1.5 h or until the bromophenol blue tracking dye reached the end of the 12.5-cm gel. The gel was then washed in three successive changes of 1.0 mM Na_2EDTA, pH 7.5, stained with ethidium bromide (2 μg/mL in water) for 30 min, and destained overnight in water. In cases where phenol extraction was employed, DNA was redissolved and electrophoresed in the absence of $NaDodSO_4$. No difference in results was detected between the two extraction techniques. Gels were calibrated with ϕX174 *Hae*III restriction fragments (BRL, Inc.).

Isolation of Multimeric Nucleosomes and Sucrose-Gradient Sedimentation. Multimeric nucleosomes were prepared by digesting nuclei in our buffer at 0 °C for either 30 min or 1 h as suggested by Strätling (1979). The digests were sedimented at 18000g for 10 min and the supernatants applied to linear 15–45% sucrose gradients containing either 150 mM or 500 mM NaCl and 0.2 mM Na_2EDTA, pH 8.0. Centrifugation in an SW 27 rotor (Beckman) was at 100000g for 16 h at 4 °C. Gradients were fractionated from the top, passed through a UV absorbance monitor (Isco, Inc.), and scanned at 254 nm. Multimer fractions were collected and dialyzed overnight at 4 °C against 150 mM or 500 mM NaCl in the presence of 0.2 mM Na_2EDTA, pH 8.0, or, in one experiment, against 0.2 mM Na_2EDTA, pH 8.0, alone. Fractions were repurified by centrifugation in an identical sucrose gradient in an SW 41 (Beckman) rotor at 200000g for 4 h at 4 °C. Purified multimeric nucleosome fractions were redigested after dialyzing overnight against the appropriate salt concentrations together with 0.2 mM Na_2EDTA, pH 8.0, at 4 °C.

Photography and Scanning. Stained gels were displayed on a shortwave UV transilluminator (Ultraviolet Products) and photographed with Polaroid Type P/N 55 film, employing a Vivitar (02) orange filter. The negatives were scanned at 560 nm in a Gilford 250 spectrophotometer equipped with a linear transport scanner. Areas corresponding to the various DNA fragments were estimated by tracing the area bounded by each peak and lines drawn vertically from the lowest points in the adjacent troughs to the bottom of the scan. Areas were then determined by weighing each silhouette and correcting for the background density of the negative.

Analysis. The weight of the silhouettes for DNA fragments in the size classes monomer–tetramer and the weight corresponding to the total ethidium-staining material on each gel were then applied to a computerized test of agreement with the theory of random scission defined by eq 1 for an infinitely long linear polymer with regularly spaced, equally accessible sites for nuclease cleavage (linkers). This test consisted of two steps. First, the weight of the silhouette corresponding to each DNA fraction was normalized to one with respect to the total, and the proportion of total DNA not included in the monomer–tetramer classes was assigned to the greater-than-tetramer class (IV<). The value of p in eq 1 which gave the best fit of the equation to each gel was then determined by using a FORTRAN program to test 10000 incremental values of p ($0 \leq p \leq 1$) throughout the entire course of theoretical random digestion. This term (p) is the percent of linkers remaining intact during the course of digestion. The "best-fit" p for all DNA size classes on an individual gel or series of gels was obtained by minimizing the square error (SE) between expected and observed values for each fraction (eq 2) where O_n

$$\mathrm{SE} = \sum_{n=1}^{5} (O_n - E_n)^2 \qquad (2)$$

is the observed proportion of total DNA in the nth size class, and E_n is the proportion expected in that class for a particular value of p in eq 1. Note that this method of estimating p, unlike methods used previously (Burgoyne & Mobbs, 1975; Lohr et al., 1977a), does not depend on the proportion of DNA in any single size class.

If the random-scission model is correct, we would not expect any *systematic* departures of the observed distributions of DNA fragments from distributions given by eq 1 with the best-fit values of p for each experiment. In other words, over a number of experiments, positive deviations (excess) and negative deviations (shortage) should occur with equal frequency within any given size class. This requirement forms the basis for the second step in our analysis, a simple χ^2 function, with four degrees of freedom, calculated as

$$\chi^2 = \frac{4}{i} \sum_{n=1}^{5} \left(D_n - \frac{i}{2} \right)^2 \qquad (3)$$

where i is the number of experiments, D_n is the number of positive deviations in the nth size class among D experiments, and the subscript n, as before, runs over five size classes. Standard χ^2 tables are used to determine alpha (α), the probability that the observed χ^2 value would occur by random (nonsystematic) deviations around the best-fit distribution given by eq 1. Significant values of α ($\alpha < 0.05$) reject the random-scission model.

Results

To ensure that the concentrations of the DNA fragments in the mononucleosome–tetranucleosome class could be reliably

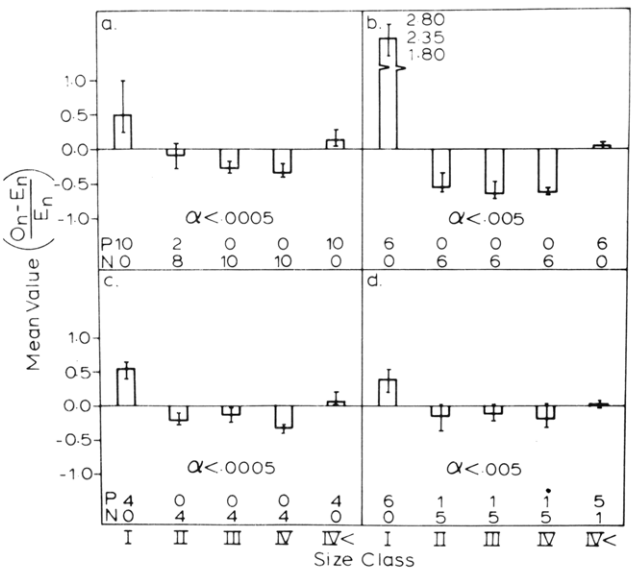

FIGURE 2: Deviations of the observed distribution of DNA fragments from the best-fit distribution allowed by the random-scission model are illustrated by plotting the quantity $(O_i - E_i)/E_i$ for each of the five sizes classes in a given series of gel profiles (O_i and E_i are defined in the text). The length of the bars indicates the size of the deviation in proportion to the expected value. Bars extending above the base line indicate positive deviations (excess), and those extending below indicate negative deviations (shortage). The range of observed values for each fragment class is represented by the vertical line (I) drawn through the mean. The probability that the observed deviations from expected values (random) arise by chance alone is given by alpha (α). Positive and negative deviations (P and N) refer to the number of gel profiles that exhibit DNA concentrations either greater than or less than the value expected for the random case in each size class. The sum of the positive and negative deviations is the number of samples used in the analysis. (a) Digestion of nuclei in the presence of 150 mM KCl. Nuclei at 6.5 A_{260} units of chromatin DNA/mL were digested, samples were taken after 10, 15, 20, 24, 25, 35, 45, and 55 min of digestion, and the DNA fragments were isolated and analyzed as described under Materials and Methods. Over the time course of digestion, mononucleosomes comprised from 20 to 36% of the total ethidium-staining material on the gel. (b) Digestion of nuclei in the absence of 150 mM KCl. Digestion conditions were the same as in (a) except nuclei were resuspended in 10 mM Pipes only. Reaction was stopped after 5 and 6 min, and the samples were treated as described above. Mononucleosomes ranged from 25 to 41% during this series of digestions. (c) Digestion of chromatin in the absence of 150 mM KCl but in the presence of Mg^{2+}. Conditions were the same as in (a) except that the digestion medium was supplemented with either 1.0 mM or 5.0 mM $MgCl_2$. Reaction was terminated after 2, 6, and 7 min, and the samples were treated as in (a). Mononucleosomes ranged from 15 to 35% over the time course of digestion. (d) Digestion of nuclei in the presence of 150 mM KCl and Mg^{2+}. Conditions were the same as in (a), but the digestion medium was supplemented with either 1.0 mM or 5.0 mM $MgCl_2$. Reaction was terminated after 50, 60, 85, and 100 min. Mononucleosomes ranged from 8 to 27% in these samples.

estimated from gel staining, photography, and scanning, identical and different loadings from the same digest halted at different extents of digestion were subjected to electrophoresis and analyzed as described under Materials and Methods. The errors involved in quantitating the DNA were found to be insignificant when compared to the deviations from randomness discussed below.

Digestion of Nuclei under Various Ionic Conditions. In order to test for random cleavage of chromatin during micrococcal nuclease digestion, chicken erythrocyte nuclei were digested to varying degrees, the DNA was displayed on gels, and the amount of DNA in each of five size classes (mononucleosome–tetranucleosome and greater-than-tetranucleosome) was measured. These results were then tested for random cleavage, as described under Materials and Methods.

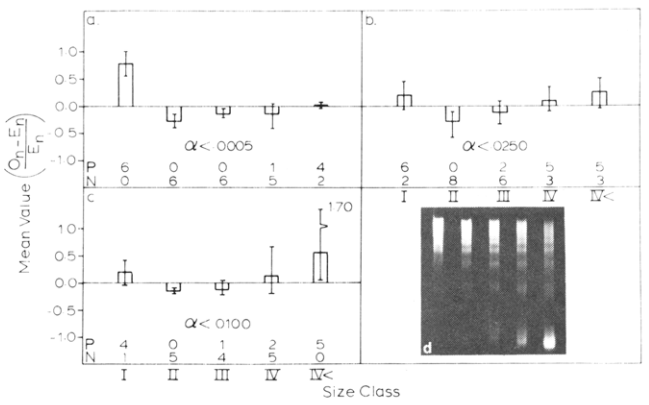

FIGURE 3: (a) Digestion of multimeric nucleosomes. Multimeric nucleosomes at 0.333 A_{260} unit/mL were redigested at 37 °C in the presence of 1.0 mM $CaCl_2$, 10 mM Pipes, and 150 mM KCl, pH 8.0, and the reaction was terminated after 1, 5, and 10 min of digestion. The samples were then treated and analyzed as usual. Mononucleosomes ranged from 10 to 22% over the time course of digestion. (b) Isolation and digestion of light multimeric nucleosomes. A multimeric nucleosome fraction of smaller size was prepared as described under Materials and Methods except that digestion was allowed to proceed for 1 h at 0 °C. Fractions on the "light" side of each peak were pooled, dialyzed, and repurified as usual, before redigestion for 1, 5, 10, and 25 min. Mononucleosomes ranged from 20 to 46% in these samples. (c) Redigestion of light multimeric nucleosomes under low-salt conditions. The multimeric nucleosome fraction isolated in Figure 3b was dialyzed against 0.2 mM Na_2EDTA, pH 8.0, alone and then subjected to redigestion as in Figure 2 except for the absence of 150 mM KCl and a reduction in Pipes to 5.0 mM. Reaction was terminated after 1, 5, 10, 25, and 60 min. (d) Electrophoretic pattern of DNA fragments in (b). Left to right: 0, 1, 5, 10, and 25 min of digestion.

Digestion conditions were varied so that the nuclear chromatin ranged from highly condensed (150 mM KCl, 5.0 mM $MgCl_2$, 1.0 mM $CaCl_2$, and 10 mM Pipes) to well dispersed (10 mM Pipes and 1.0 mM $CaCl_2$). The results of these experiments are presented as histograms, showing the magnitude of deviations from that expected for random cleavage by using the best-fit distribution derived from eq 1 (Figure 2a–d).

Digestion of intact nuclei in the presence of 150 mM KCl (Figure 2) is distinctly nonrandom as our degree of confidence in rejecting the random case is greater than 99%. In addition, the histograms reveal that, relative to the random model, the observed concentrations of monomer and DNA fragments of size greater than tetramer are larger than expected (positive deviations) whereas the observed quantities of dimer–tetramer fragments are less than expected (negative deviations). This pattern is also found throughout the remaining experiments, including those conducted in the absence of KCl (Figure 2b), and appears to be characteristic of digestion of whole chromatin. We can also reject randomness in the digestions without KCl but in the presence of Mg^{2+} (Figure 2c) and for digestions plus both salts (Figure 2d). These results indicate that variations in ionic conditions that affect the state of aggregation and conformation of chromatin fibers (Sanders, 1978; Thoma et al., 1979; Finch & Klug, 1976) have no effect on the pattern of nonrandom cleavage by micrococcal nuclease.

Digestion of Multimeric Nucleosomes under Various Ionic Conditions. Multimeric nucleosomes (average size 25 nucleosomes), prepared according to Materials and Methods and checked for integrity by electron microscopy, were redigested with micrococcal nuclease to ascertain whether the same nonrandom pattern observed with whole chromatin also characterized smaller size segments removed from possible steric constraints imposed by large-scale structures of chromatin. These results are presented in Figure 3a–d. Analysis of the digestion of the multimeric nucleosome fraction in

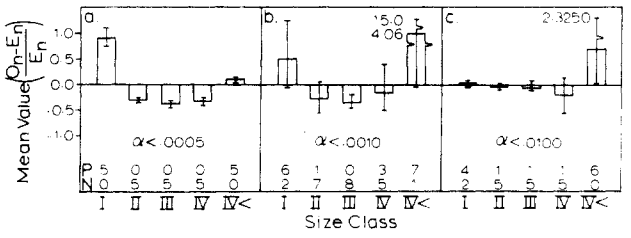

FIGURE 4: (a) Analysis of DNA fragments resulting from micrococcal nuclease digestion of chicken erythrocyte nuclei by Woodcock and Sweetman (C. L. F. Woodcock and H. E. Sweetman, unpublished experiments). (b) Analysis of radiolabeled DNA fragments from micrococcal nuclease digestion of interphase and metaphase chromatin in CHO cells by Munroe & Latt 1977; their Table II). (c) Analysis of observed weight fractions from gels after digestion of yeast nuclear chromatin by Lohr et al. (1977b; their Figure 3).

Figure 3a once again indicates that random cleavage for oligonucleosomes of this size can be rejected. In addition, deviations from expected values follow the general pattern previously observed with whole chromatin. However, when a multimeric fraction of smaller size (15 units) is used as substrate (Figure 3b), the *pattern* of deviation from randomness is similar to those of the larger multimer and whole chromatin, but in this case the *extent* of the deviations is reduced. A similar pattern was seen when the same oligonucleosome fraction was dialyzed exhaustively against 0.2 mM Na_2EDTA prior to redigestion under low-salt conditions Figure 3c.

Preliminary experiments have been performed with multimer nucleosomes largely depleted of histones H1 and H5 by washing with 500 mM NaCl. Digestion of this fraction exhibited the same general pattern of deviations from randomness. We therefore tentatively conclude that the features of nonrandomness we have observed are not altered by removal of H1 and H5.

We have also examined deviations throughout the course of individual digestions in order to determine whether the observed departures from randomness vary with the extent of digestion. When the data are grouped according to the extent of digestion, we find statistically significant nonrandom patterns ($\alpha < 0.0005$) for all groups (data not shown).

In view of these results, we decided to examine previous published and unpublished data to see if similar nonrandom effects were seen. We have analyzed electrophoretic patterns of DNA fragments from digestions of chicken erythrocyte nuclei in prior experiments from this laboratory (C. L. F. Woodcock and H. E. Sweetman, unpublished experiments) and those resulting from digestion of interphase and metaphase chromatin in CHO cells published by Munroe & Latt (1977), and presented in tabular form, and observed weight fractions of DNA fragments produced from digestion of yeast chromatin by Lohr et al. (1977a). The results are given in Figure 4a–c. Although randomness can be rejected in these samples on the basis of the χ^2 test, it should be noted that in some cases, e.g., Figure 4b, the range of observed values was often rather large. However, the pattern of deviation from randomness was similar to that observed in the chicken erythrocyte system. Other published data were also analyzed, with similar results.

Discussion

Analysis of the products of micrococcal nuclease digestion of chromatin indicates that the process is not a completely random one. It should be realized, however, that the present experiments are based on measurements of the total number of fragments in the various size classes, and consequently do not address other aspects of randomness such as the location of core particles along the DNA fiber (Prunell & Kornberg, 1978; Baer & Kornberg, 1979; Lacy & Axel, 1975), variations in linker DNA length (Lohr & Van Holde, 1979; Lohr et al., 1977b,c), or differential sensitivity of specific genes (Johnson et al., 1978; Reeves, 1978; Tata & Baker, 1978; Bloom & Anderson, 1979; Bellard et al., 1978).

Since nonrandom cleavage was unexpected, we have carefully examined our methodology and assumptions for possible errors. It seems unlikely that we are consistently underestimating the dimer–tetramer region on gels and overestimating monomer and all DNA fragments larger than the tetramer size. It is possible that the accumulation of submonomer breakdown products would affect the results, and we have therefore avoided analysis of gels in which digestion had proceeded to the point where submonomer products were appreciable.

Another point of concern is that the systematic differences observed are the result of inaccuracies in the quantitation of DNA on the gels by ethidium bromide staining (Pulleyblank et al., 1977). We have tested this possibility by running gels with different DNA loadings that ranged over the concentrations used in our experiments. We find a linear response between the amount of total DNA applied and the quantity measured on the gel in the various DNA size classes, and we have shown that the small experimental error in these measurements cannot account for the systematic deviations from randomness which we observe. Furthermore, as pointed out by Levinger & Carter (1979), the errors in quantitation from ethidium bromide scans (Pulleyblank et al., 1977) are minimal in the early stages of digestion (10–30% mononucleosomes) where most of our data was obtained. In addition, there is no evidence to support differential uptake of ethidium by the various DNA fractions, except on the basis of size, or its preferential loss during the destaining step in water. Gels scanned almost immediately after staining do not differ in the cleavage pattern from those destained in water overnight, except for the presence of a greater background in the former. This also indicates that DNA fragments at least as large as the monomer range we have measured remain in the gel throughout the destaining process. Finally, Munroe & Latt (1977) have used radioactivity to quantitate DNA fragments resulting from various times of digestion of chromatin. As noted in Figure 4b and discussed earlier, their values indicate a nonrandom process with features similar to those resulting from our own experiments. Thus, an independent DNA measuring system produces similar values to those determined with ethidium staining.

The ability of the χ^2 test to distinguish between the random and nonrandom modes of nucelase cleavage is, of course, dependent on the number of samples analyzed. We have therefore emphasized, thoughout all experiments, inspection of *patterns* of devation from randomness, that is, magnitude, direction, and the number of positive and negative deviations, together with the χ^2 result.

The results of the present studies offer evidence that micrococcal nuclease cleavage of linker DNA regions in whole chicken erythrocyte chromatin proceeds in a nonrandom fashion regardless of the ionic conditions. It might have been expected that the pattern would be different when comparing digestions of native chromatin in the intact nucleus with chromatin fibers dispersed in low ionic strength media.

However, experiments performed in the presence and absence of 150 mM KCl (Figure 2a–d) clearly show that this is not the case. The presence of divalent cations, which mimic the effects of monovalent salt on chromatin conformation but at much lower concentrations (Bradbury & Carpenter, 1973),

also did not alter the digestion pattern. The concentration of Mg^{2+} used in these experiments has been shown to enhance aggregation of chromatin fibers and to preserve the characteristic condensation distribution found in nuclei, an affect also displayed by Ca^{2+} (Sanders, 1978; Thoma et al., 1979; Bradbury & Carpenter, 1973; Olins & Olins, 1972; Finch & Klug, 1976). Since Ca^{2+} is required for micrococcal nuclease activity, the effect of this cation was not examined.

The digestion patterns of oligonucleosome fragments closely resembled the nonrandom cleavage found in whole chromatin, and, hence, it would appear that the structural or conformational properties that lead to this pattern of cleavage are not the result of some large-scale organization of chromatin. However, when a smaller size oligonucleosome fraction was used as substrate, the extent of deviations from randomness was smaller, even though the *pattern* of deviations from randomness was similar to those of larger oligomer and whole chromatin. Again, ionic conditions did not affect the results: digestions of a small oligonucleosome fraction under low-salt conditions (Figure 3c) or in the unusual ionic strength buffer (Figure 3b) both display the same features of nonrandomness as previously described for whole chromatin. This indicates that the same insensitivity to ionic conditions also characterizes smaller chromatin segments of this size.

Digestion of an oligonucleosome fraction depleted in histones H1 and H5 exhibited a similar pattern of nonrandomness. Therefore, we can tentatively conclude that histones H1 and H5, which are known to be involved in conferring higher order structure of chromatin (Strätling, 1979; Brach, 1976; Thoma et al., 1979; Sahasrabuddhe & Van Holde, 1974; Keller et al., 1978; Griffith & Christiansen, 1978; Oudet et al., 1975; Whitlock & Simpson, 1976; Lawson & Cole, 1979; Renz et al., 1977; Thoma & Koller, 1977; Christiansen & Griffith, 1977; Varshavsky et al., 1977; Spadafora et al., 1979) and are located, at least in large part, in the linker region (Noll & Kornberg, 1977; Bakayev et al., 1977; Gaubatz & Chalkley, 1977; Varshavsky et al., 1976), are not directly responsible for nonrandom cleavage. It should be noted, however, that the 500 mM NaCl treatment used to prepare this fraction is also sufficient to remove a majority of nonhistone proteins (Johns, 1971).

Analysis of results from the studies of Munroe & Latt (1977), Lohr et al. (1977a), and previous experiments in this laboratory as well as profiles from other published reports (Figure 4a–c) suggests that chromatins of different transcriptional activity and condensation exhibit the same type of deviation from randomness.

Our results are at variance with those of Burgoyne & Mobbs (1975). These authors based their conclusion of random cleavage by endogenous endonuclease in rat liver chromatin on the apparent agreement in probabilities of cleavage between the weight fraction of monomer DNA fragments and all fragments greater than dimer as a single group. Dimeric fragments were excluded in the determination of cleavage probability because such values generally disagreed with those obtained for the other two classes. This observation was attributed to the difficulty in estimating p for dimer in the region of the theoretical random plot where the weight fraction of this component changes very little compared to a large change in p values. When we analyze our data according to their method, we also obtain close agreement in p values, suggesting that their method of analysis is insufficiently sensitive.

In their study of the pattern of micrococcal nuclease digestion in yeast, Lohr et al. (1977a) used the monomeric DNA concentrations alone to derive p, and hence the expected weight fractions for other oligomers in a given digestion. They found that observed and expected quantities were in close agreement and concluded that random cutting occurs. Treatment of our erythrocyte data in an identical manner discloses nonrandom cleavage with a similar pattern of deviations to those already described in this report, showing that the two methods give comparable results in cases where deviations from randomness are quite pronounced. When we analyzed some of the data of Lohr et al. (1977a) by our method, we obtained small deviations from randomness (Figure 4c) which again showed the pattern of excess monomer and IV< and shortages in II, III, and IV characteristic of other chromatin samples. The distribution of yeast DNA fragments most closely approximates the random case and must reflect a fundamental property of yeast chromatin that distinguishes it from the other samples examined.

One interpretation of our findings is that once a chromatin fiber is cut or a polynucleosome segment first excised (Hozier et al., 1977; Strätling et al., 1978; Butt et al., 1979) it is preferentially digested from the ends to yield monomers. This mechanism would give rise to larger than expected quantities of monomer and material of high molecular weight (IV<). We had predicted that such a mechanism would be enhanced under conditions where chromatin was condensed, and the nuclease perhaps prevented from access except at the free ends. Although this prediction was not fulfilled (Figure 2a–c), a preferential cutting of free ends seems to be the most likely way to generate excess mononucleosomes. Alternatively, the linker DNA regions of small oligomers such as dimers, trimers, and tetramers may be cleaved more rapidly than higher oligomers to give the observed pattern of deviation from randomness. In this case, the linker regions of small oligomers would be inherently more susceptible to nuclease cleavage than those of larger multinucleosomes. Although the mechanism involved in nonrandom cutting is undetermined, the phenomenon itself seems to be well established, and could be an important factor in interpreting results of experiments in which chromatin is probed with this nuclease. We plan to extend this approach to examine the patterns of cleavage for specific gene sequences.

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