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Interaction of nucleosome core particles with distamycin and echinomycin: analysis of the effect of DNA sequences

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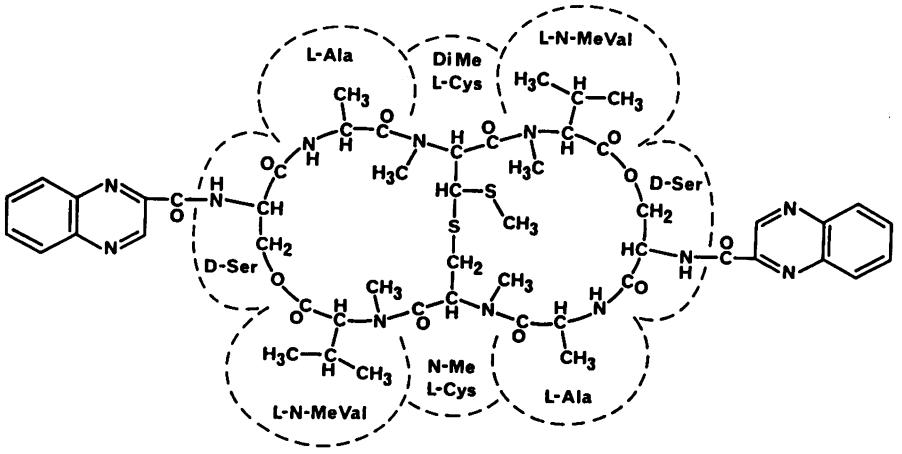
ABSTRACT

Two fragments of Xenopus borealis DNA 135 and 189 base-pairs long were separately incorporated into nucleosome core particles by reconstitution with chicken erythrocyte histones, and incubated with echinomycin (a bis-intercalating antitumour antibiotic) or distamycin (a minor groove-binding, non-intercalating antibiotic). Controlled digestion of these defined sequence core particles using DNAase I revealed new cleavage products, indicative of a change in orientation of the DNA molecule on the surface of the nucleosome. This new rotational setting of DNA within the core particle induced by antibiotic binding appears to be practically independent of DNA sequence, although some differences were noted between the patterns of fragments observed in the various experiments, most likely reflecting the exact number and disposition of the antibiotic binding sites.

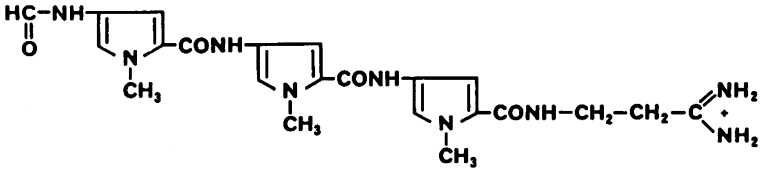
INTRODUCTION

We have recently shown [1,2] that when defined-sequence nucleosome core particles containing the tyrT DNA fragment are exposed to several antibiotic ligands, the DNA rotates with respect to the histone octamer by about half a turn. In the presence of the bis-intercalating antibiotic echinomycin, as well as the narrow groove-binding ligands distamycin, berenil or netropsin, new DNAase I digestion products appear positioned approximately mid-way between those characteristic of control core samples. These results have been provisionally explained by suggesting that the changes in rotational orientation of the core DNA serve to optimize non-bonded contacts between the ligand and the polynucleotide backbone [1,2].

Since all the previous observations were made with the same piece of DNA (the tyrT fragment) we must question whether the observed rotation is an inherent consequence of antibiotic binding or whether it is explicable on the basis of some peculiar property of this DNA fragment bent around the histone octamer [3]. The tyrT fragment is, after all, of prokaryotic origin and presumably not 'intended' to adopt a strictly phased position within the nucleosome. As regards natural eukaryotic DNA it has been suggested that



Echinomycin



Distamycin A

Figure 1 Chemical structures of echinomycin and distamycin.

certain sequence-dependent modulations in structure determine the phasing, that is the positioning of DNA on the core particle [3,4]. Nevertheless, in general the features observed with nucleosome cores reconstituted using the tyrT DNA fragment [3] have been shown to apply to nucleosome cores reconstituted with other DNA fragments [4], suggesting that there is nothing particularly unusual about tyrT DNA in this respect.

In this paper we study the interaction of echinomycin and distamycin (Figure 1) with nucleosome core particles containing two different restriction fragments obtained from plasmid pXbs1, which contains a Xenopus borealis gene for 5S ribosomal RNA [5] (Figure 2). Our results show that each antibiotic causes the DNA to rotate on the surface of both these nucleosome cores (referred to as "core 1" and "core 5" in [4]). There are, however, certain significant differences between the behaviour of the two DNA fragments. They

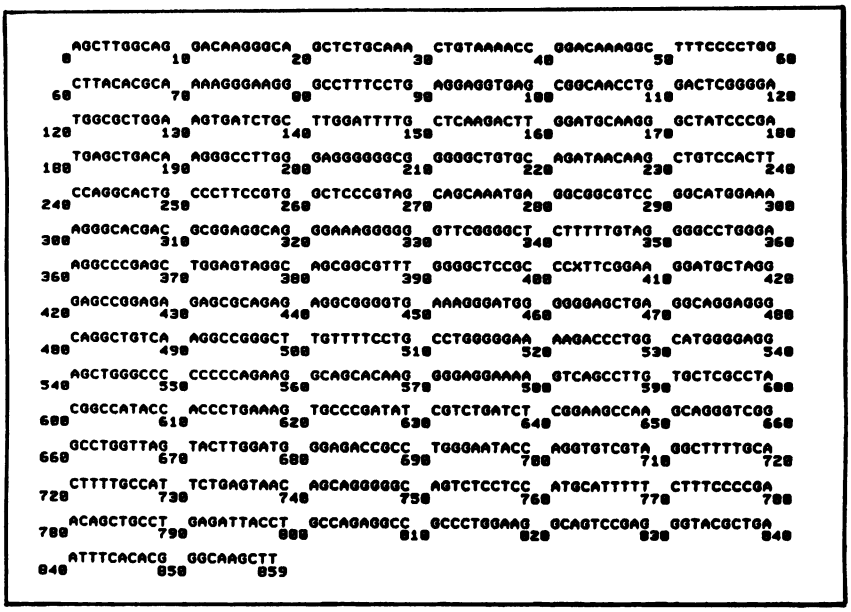


Figure 2 Sequence of a Xenopus borealis DNA fragment (after [4]). This DNA molecule, from plasmid pXbs1, contains one copy of a gene for 5S RNA, the synthesis of which begins after position 595 and ends after position 714 [5]. The Sau3AI (1-135) and ScaI (671-859) restriction fragments were used in the reconstitution experiments.

were chosen so as to provide examples of a strongly phased sequence (the 135-mer, incorporated into "core 1" of [4]) and a poorly phased sequence (the 189-mer, incorporated into "core 5"). We have attempted to analyse these observations in some detail, so as to assess the relationship between induced rotation of nucleosome core DNA and the number and position of potential antibiotic binding sites.

MATERIALS AND METHODS

Antibiotics

Echinomycin was a gift from Drs. H. Bickel and K. Scheibli of CIBA-Geigy Ltd., Basel, Switzerland. Distamycin hydrochloride was a gift from Dr. F. Arcamone, Farmitalia, Italy. Solutions of both antibiotics were prepared as described in [1].

Preparation of the 135-mer and 189-mer

Plasmid pXbs1 is a derivative of pBR322 containing a Xenopus borealis somatic 5S RNA gene inserted into the unique HindIII site [5]. The 860 base-

pair DNA insert was cut from the plasmid with HindIII and 3'-end labelled using reverse transcriptase and [α-^{32}P]dATP. This 860 bp piece of DNA (Figure 2) was separated from the rest of the plasmid on a 20cm non-denaturing 5% polyacrylamide gel [4] and further digested with Sau3AI and ScaI. The resulting fragments were then subjected to electrophoresis on a 40cm non-denaturing 6% polyacrylamide gel. This yielded two 3'-labelled fragments: the 135-mer (nucleotides 1-135, Sau3AI fragment) bearing the 3'-label on the bottom strand and the 189-mer (nucleotides 671-859, ScaI fragment) labelled on the top strand.

Preparation and digestion of DNA-core complexes

The 135-mer and 189-mer DNA fragments were each incubated separately with a 100-fold molar excess of nucleosome core particles from chicken erythrocytes (a gift from Drs. H.R. Drew and D. Rhodes) for 20 mins at 37°C in a solution containing 20mM Tris-HCl pH 7.8, 800mM NaCl, 0.2mM EDTA and 0.2mM PMSF. The final salt concentration was then lowered to 70mM NaCl as described in [3].

Digestion of the reconstituted material with DNAase I was performed in parallel with a free DNA control, which had been subjected to an identical procedure omitting the nucleosomes. In a typical experiment 20µl of core complex (or of the free DNA control) were incubated with an equal volume of antibiotic solution and subjected to DNAase I digestion at an enzyme concentration of 5.0 units/ml for core and 1.0 unit/ml for free DNA. The reaction mixture was extracted and recovered as previously described [1-3] and fractionated by electrophoresis on an 8% denaturing polyacrylamide gel containing 8M urea. Bands in the digests were assigned by reference to dimethylsulphate/piperidine markers specific for guanine [6].

Densitometry

Autoradiographs of the gels were scanned using a Joyce-Loebl microdensitometer to produce profiles from which the relative intensity of each band was measured. The data were then expressed in the form of fractional cleavage (f) = A_i/A_t where A_i is the area under band i and A_t is the sum of the areas under all bands in any gel lane [1,2,7]. Plots of DNAase I cleavage inhibition are presented in the form ln ($f_{antibiotic}$) - ln ($f_{control}$) representing the differential cleavage at each bond relative to that in the control. The results are displayed on a logarithmic scale for the sake of convenience in plotting so that positive values indicate enhanced cleavage whereas negative values indicate blockage.

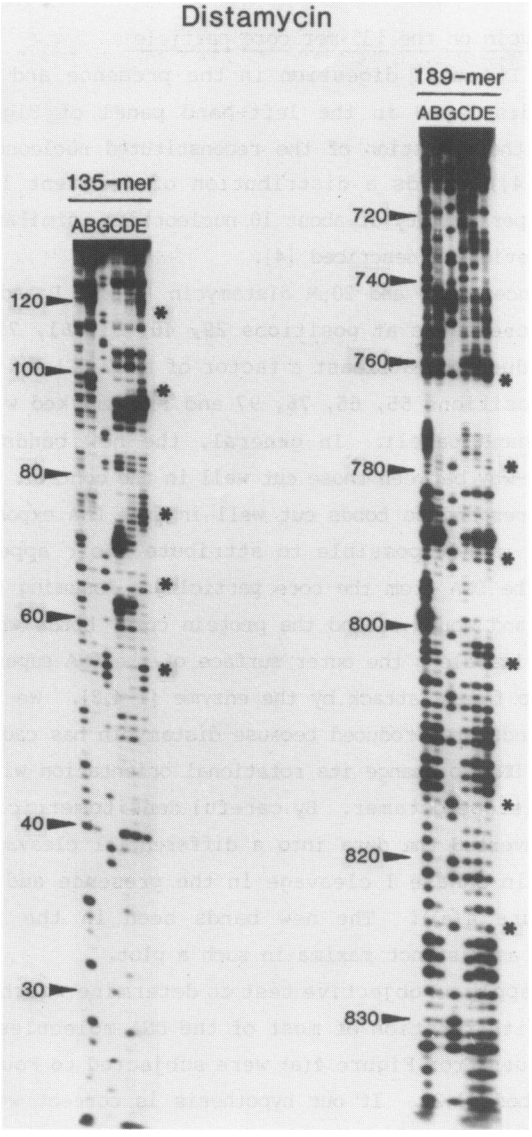


Figure 3 DNAase I footprinting of distamycin bound to free DNA and its effect on DNAase I digestion of reconstituted nucleosome core particles. Electrophoretic patterns for the 135-mer DNA fragment (left-hand panel) and 189-mer DNA fragment (right-hand panel) are shown. Lanes are labelled as follows: G for chemical cleavage at guanine nucleotides; A for free DNA; B for free DNA plus 10μM antibiotic; C for control core particles; D for core plus 10μM antibiotic; E for core plus 20μM antibiotic. Asterisks indicate the positions of new bands which appear in digests from core particles treated with the antibiotic.

RESULTS

Effects of distamycin on the 135-mer core particle

Patterns of DNAase I digestion in the presence and absence of this antibiotic are displayed in the left-hand panel of Figure 3. With no distamycin added the digestion of the reconstituted nucleosomes (referred to as "core 1" in [4]) yields a distribution of fragment lengths which is modulated with a periodicity of about 10 nucleotides, similar in all respects to the pattern previously described [4].

In the presence of 10 and 20μM distamycin (tracks D and E) the intensity of the control core bands at positions 29, 40, 51, 61, 71, 91 and 110 is progressively reduced by at least a factor of two. Clear new bands appear located around positions 55, 65, 76, 97 and 119 (marked with asterisks in Figure 3, left-hand panel). In general, the new bands lie in regions approximately mid-way between those cut well in the control. However, they do not generally correspond to bonds cut well in free DNA exposed to distamycin (track B), so it is not possible to attribute their appearance to gross displacement of the DNA from the core particles. Assuming then that the DNA remains attached and wound around the protein core, bonds which are cut well by DNAase I must lie along the outer surface of the DNA supercoil, where they will be exposed to facile attack by the enzyme [1-4,8]. We therefore suggest that these new bands are produced because distamycin has caused a substantial proportion of the DNA to change its rotational orientation with respect to the surface of the histone octamer. By careful densitometric analysis of each lane, we have converted the data into a differential cleavage plot depicting the differences in DNAase I cleavage in the presence and absence of 20μM distamycin (Figure 4(a)). The new bands seen in the presence of the antibiotic appear as distinct maxima in such a plot.

In order to apply an objective test to determine whether these results are consistent with rotation of most of the DNA molecules by about half a turn, all the values from Figure 4(a) were subjected to Fourier analysis as previously described [1,2]. If our hypothesis is correct we would expect to find a strong periodicity of about 10 bp.

The results of the computer analysis are recorded in Table 1. There is indeed a strong, regular variation in the difference plot with a period of 10.42 nucleotides, which is identical to the periodicity calculated for core particles reconstituted with tyrT DNA and exposed to distamycin [1]. No other significant periodic variations modulated within the range 5.0 to 19.0 bonds were detectable. The maxima calculated in the Fourier analysis (Table 1)

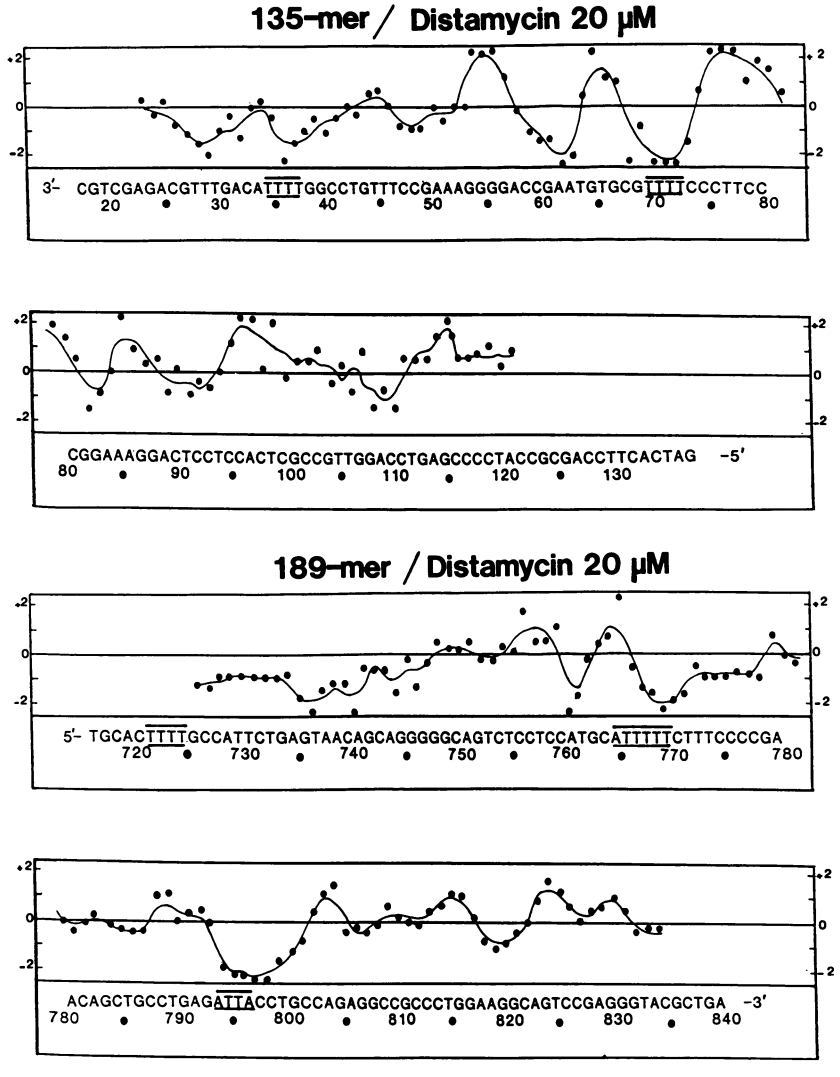


Figure 4 Differential cleavage plots representing the effect of 20μM distamycin on the susceptibility of nucleosome core particles to DNAase I cleavage. (a) Effect on digestion of the "Crick" (lower) strand of the 135-mer core which reads 5' to 3' right-to-left. (b) Effect on digestion of the "Watson" strand of the 189-mer core which reads 5' to 3' left-to-right. Vertical scales in both diagrams are expressed in units of ln (f_a) - ln (f_c), where f_a is the fractional cleavage at any bond in the presence of antibiotic and f_c is the fractional cleavage of the same bond in the control, for closely similar extents of overall digestion. Positive values indicate enhancement, negative values blockage. The smooth line is a three-bond running average calculated by averaging the value at any bond with those of its two nearest neighbours.

Table 1 Fourier analysis of the differences in digestion between nucleosome core particles exposed to 20μM antibiotic and control cores.

Antibiotic	Amplitude	Period (bp)	Positions of maxima
135-mer core particle			
Echinomycin	26.0	10.42	26.4,36.8,47.2,57.7, 68.1,78.5,88.9,99.3, 109.8,120.2,130.6
Distamycin A	47.1	10.42	24.4,34.8,45.2,55.7, 66.1,76.5,86.9,97.3, 107.7,118.2,128.6
189-mer core particle			
Echinomycin	23.0	10.87	734.2,745.1,755.9, 766.8,777.7,788.6, 799.5,810.3,821.2
Distamycin A	22.7	12.20(*)	741.0,753.2,765.4, 777.6,789.8,802.0, 814.2,826.4

Amplitude represents the maximum amplitude for any Fourier wave spanning the region of 5.0 to 19.0 base pairs. Period is the value of Fourier periodicity at the position of maximum amplitude.
(*) This value could reflect the existence of detached DNA at either or both ends of the core particle. See the text for a detailed discussion.

agree nicely with the experimental results shown in Figures 3 and 4(a).

Distamycin binds to AT-rich regions in the DNA minor groove without intercalating between base pairs [9-11]. Two potential specific binding sites are located in the vicinity of positions 36 and 71 in the "135-mer" (Figure 2). When this DNA is incorporated into nucleosome core particles the zone near position 71 behaves as if it faces away from the protein surface whereas that around position 36 is positioned on the inward-facing surface of the polynucleotide supercoil [4]. After the addition of 20μM distamycin A both positions appear strongly protected from DNAase I cleavage. Protection of the region around position 71 is attributable to the induced rotation as well as antibiotic binding, since this sequence ends up facing inward towards the histone octamer, but the protection around position 36 must result from the effect of distamycin binding alone since it now faces outwards. Indeed, it is noteworthy that the Fourier analysis (Table 1) predicts the occurrence of a distinct maximum close to position 35 at the left-hand end of this binding

site, yet the experimental results (Figure 4(a)) show that this predicted peak is largely suppressed by the direct protection effect centred around position 36.

Because distamycin does not substantially alter the periodicity of the double helix in either free or core DNA, it is not possible to provide a direct estimate of the true number of antibiotic molecules bound per core particle. However, the presumptive evidence of binding to the two strong sites near positions 36 and 71 means that the average level of binding is likely to be at least two distamycin molecules per nucleosome core.

Effects of distamycin on the 189-mer core particle

Nucleosome core particles are reported to contain 146 (±2) base-pairs of DNA [12]. When a longer piece of DNA is reconstituted together with histones H2A, H2B, H3 and H4, the positioning of the core particles is non-random, so that it is possible to observe the existence of DNA bound to the histone octamer as well as naked internucleosomal ("linker") DNA [4]. This is the case with nucleosome core particles constructed using the 189-mer as described above, referred to as "core 5" by Drew and Calladine [4]. These and other authors employed micrococcal nuclease digestions [4,13] to obtain evidence concerning the location of nucleosome cores, whereas DNAase I was used to sense the rotational setting of DNA with respect to the protein octamer [3,8]. From the data of Drew and Calladine [4] we can assume that the dyad axis of this nucleosomal core particle is located circa position 750 (±20).

In the absence of added ligand (track C in Figure 3, right-hand panel) digestion of this core particle does not yield a clearly visible periodicity. However, one can observe a series of "split" peaks at approximate positions 748/752, 759/764, 777, 792/797, 807/812, which are explained by the absence of a single DNA rotational setting [4].

When distamycin, 10 or 20μM, is added to these cores new bands appear around positions 765, 779, 792, 804, 816 and 824 (Figure 3, right-hand panel), all lying several bonds away from regions which were previously cut well in the control, and again distinct from bands which are strong in free DNA exposed to the antibiotic (track B). Because of the peculiar DNAase I cutting profile in the control cores it is not possible to determine precisely the relationships between the new bands and the original "split" peaks. However, we note that once more the conspicuous new bands often lie mid-way between regions of strong cutting in the native core particles. A presumptive change in rotational orientation is most evident in the zone between bonds 778 and 824.

In the presence of either 10 or 20μM distamycin the bottom part of the footprinting pattern (bonds 825 to 840) looks very much like free DNA (see Figure 3, right-hand panel). It seems that with this fragment the antibiotic has an additional effect in perturbing the stability of the DNA ends with respect to the core particle. This behaviour is quite different from that observed with both tyrT and 135-mer DNA cores, and probably reflects the number and distribution of the AT-rich regions in the 189-mer core particle. It is known that sequences like AAA/TTT prefer to be at the very edge of the nucleosome core [14]. In the 189-mer such sequences, which are also potential binding sites for distamycin, are found in the vicinity of positions 715, 723, 767, 795 and 842 (see Figures 2 and 4(b)). The positioning of these sites about the core particle depends on the low degree of order of this particle as regards its rotational setting [4]. However, if we accept the evidence [4] that the dyad axis is located circa 750 (±20) then the only one of these regions lying outside the core particle is the one around position 842.

After interaction with distamycin all these sequences are protected from DNAase I cleavage. Consequently we cannot absolutely exclude the possibility that it would be correct, in this case, to interpret our results as a mixture of unmodified cores together with displaced free DNA. However, because the central part of the digestion pattern retains a core-like appearance we favour the idea that there is partial detachment of DNA at the ends of the core. This is most probably caused by strong antibiotic binding in the vicinity of 842 and 795, close to the edge of the particle.

A differential cleavage plot for the 20μM distamycin data is shown in Figure 4(b). When these data were subjected to Fourier analysis we obtained a periodicity of 12.20 base pairs (Table 1). This seems an unlikely value for an intact nucleosome core particle, but could be explained by the presence of several peaks present in the detached DNA segment(s) affecting the value characteristic of the central part of the core particle, which is still preserved. At all events, the finding that most sequences containing runs of four (or more) AT bases are protected by 20μM distamycin suggests that the core particle can accommodate 3-4 molecules of antibiotic, and perhaps one or two more interacting with the naked (linker) DNA.

Effects of echinomycin on the 135-mer core particle

In Figure 5 (left-hand panel) are shown patterns of fragments deriving from 135-mer cores treated with 10 or 20μM echinomycin. Conspicuous new bands appear located around positions 27, 36, 49, 59, 82, 97 and 110 (marked with asterisks) and at the same time several of the control core bands are

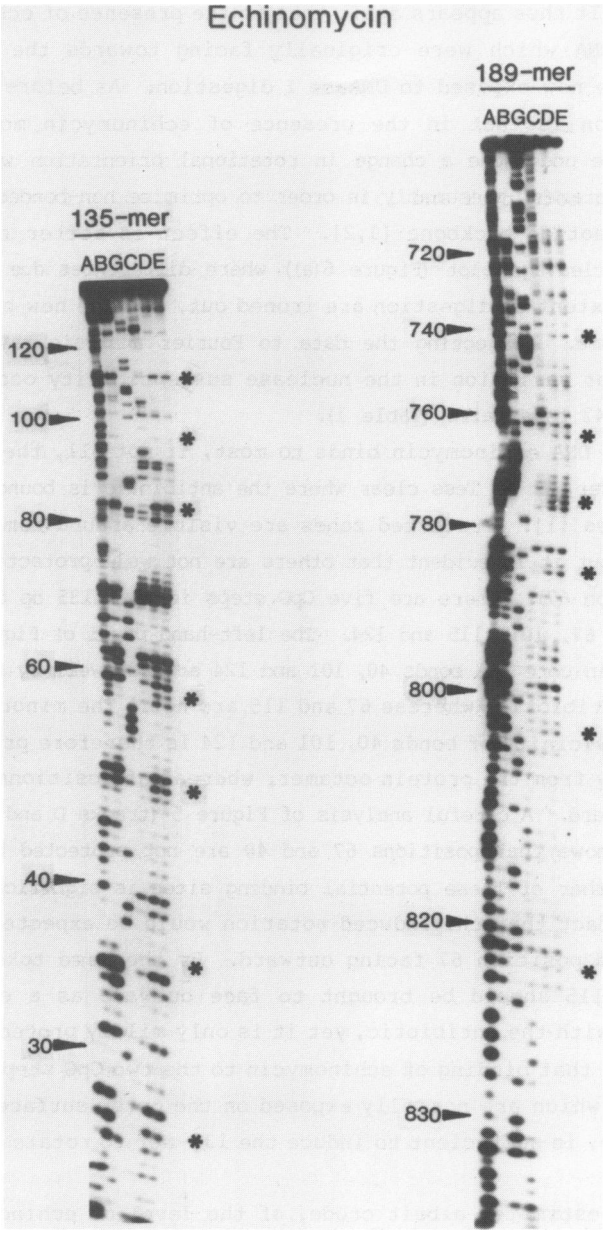


Figure 5 DNAase I footprinting of echinomycin bound to free DNA and its effect on DNAase I digestion of reconstituted nucleosome core particles. Electrophoretic patterns are shown for the 135-mer DNA fragment (left-hand panel) and 189-mer DNA fragment (right-hand panel). Details and labelling of tracks as described in the legend to Figure 3.

suppressed. It thus appears again that in the presence of echinomycin many regions of DNA which were originally facing towards the inside of the supercoil are now exposed to DNAase I digestion. As before, the simplest interpretation is that in the presence of echinomycin most of the DNA molecules have undergone a change in rotational orientation with respect to the histone octamer, presumably in order to optimise non-bonded contacts with the polynucleotide backbone [1,2]. The effect is better evidenced in a differential cleavage plot (Figure 6(a)) where differences due to unequal gel loading and extent of digestion are ironed out, and the new bands appear as distinct maxima. Subjecting the data to Fourier analysis revealed a single strong regular variation in the nuclease susceptibility occurring with a period of 10.42 base-pairs (Table 1).

On naked DNA echinomycin binds to most, if not all, the CpG sequences [7,15]. However, it is less clear where the antibiotic is bound to nucleosome core particles [1]. Protected zones are visible around some CpG steps in Figure 6(a), but it is evident that others are not well protected (for example around position 67). There are five CpG steps in this 135 bp DNA fragment at positions 40, 67, 101, 115 and 124. The left-hand panel of Figure 5 (track C) reveals that in core DNA bonds 40, 101 and 124 are cut well by DNAase I in the absence of antibiotic, whereas 67 and 115 are not. The minor groove of the helix in the vicinity of bonds 40, 101 and 124 is therefore presumed to face outward, away from the protein octamer, whereas at positions 67 and 115 it must face inward. A careful analysis of Figure 5 (tracks D and E in the left-hand panel) shows that positions 67 and 40 are not protected by echinomycin. Evidently neither of these potential binding sites is significantly occupied, despite the fact that the induced rotation would be expected to leave the region around position 67 facing outward. By the same token, the region around bond 115 should be brought to face outward as a consequence of interaction with the antibiotic, yet it is only mildly protected. It seems likely, then, that binding of echinomycin to the two CpG steps at positions 101 and 124, which are normally exposed on the outer surface of DNA in the core particle, is sufficient to induce the 135-mer to rotate on the histone octamer.

Another estimate, albeit crude, of the level of echinomycin binding required to induce the observed rotation can be derived from the alteration in helical periodicity. In the presence of the antibiotic the relative positions of maxima in the core digestion plot differ by (121-37) = 84 bonds over 8 periods, as compared with 83 bonds in control cores not exposed to any ligand

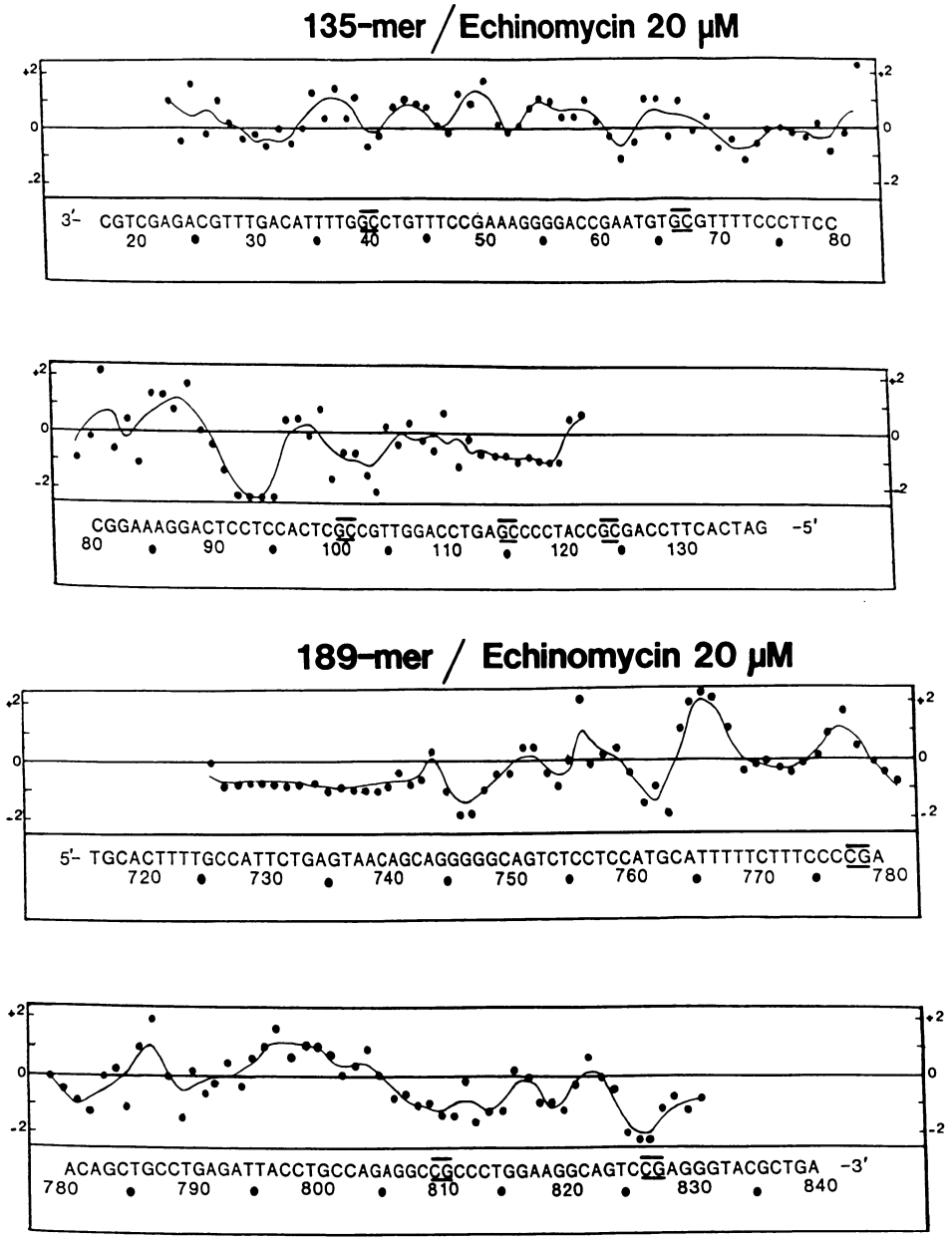


Figure 6 Differential cleavage plots representing the effect of 20μM echinomycin on the susceptibility of nucleosome core particles to DNAase I cleavage. (a) Effect on digestion of the 135-mer core particles; (b) effect on the 189-mer core particle. Presentation and details as described in the legend to Figure 4.

[4]. Hence binding of echinomycin has unwound the DNA by roughly one base-pair, or 36°. Since the acknowledged helix-unwinding angle is 48° per molecule of echinomycin which bis-intercalates [7,16], the average level of echinomycin bound per nucleosome core particle appears to be only about one. This is much lower than the estimate of 2.25 molecules calculated for the complex between echinomycin and cores containing tyrT DNA [1]. The difference most probably reflects the different number and distribution of CpG binding sites present in the respective DNA fragments.

Effects of echinomycin on the 189-mer core particle

Patterns of DNAase I digestion for this reconstituted core particle in the presence of 10 and 20μM echinomycin are shown in Figure 5 (right-hand panel). In the presence of 20μM echinomycin new bands are visible at positions 743, 765, 776, 786, 804 and 822 which appear as peaks of increased sensitivity in a differential cleavage plot (Figure 6(b)). Most of the native bands are suppressed by at least 50%, except the one circa 777 where enhanced cutting is evident, presumably as a consequence of echinomycin binding to the nearby CpG step or else a change in gross rotational orientation of the DNA as seen with the other core-antibiotic complexes. We favour the latter explanation, though the situation is manifestly more complicated than was seen with core particles containing tyrT DNA [1] or the 135-mer described above.

With the 189-mer core particles it is not possible to assess unambiguously how the new bands which appear in the DNAase I cleavage pattern in the presence of antibiotic are related to the original ones, since the result would depend on which of the original "split" peaks [4] we consider in the calculations.

Because only about 146 bp of DNA can be bent around the histone octamer [12] we can calculate that if the dyad axis lies at position 750 the protein interacts roughly between positions 677 and 823. However, if we estimate the dyad at position 770 (which is possible since micrococcal nuclease digestion only defines the particle axis within ±20 bonds [4]), the polynucleotide is bent between positions 697 and 843. In the first case, four echinomycin binding sites (CpG) are present on the core particle (688, 707, 778, 810) and one (828) is positioned close to the end of the superhelix. In the second case we also find four potential binding sites on the core (707, 778, 810, 828). We will not consider the other possible dyad axis at 730 since that would position the region in the vicinity of 810 outside the core particle, which would be inconsistent with the observed regular periodicity in this region for one set of "split" peaks [4].

The results for the interaction of echinomycin with the core particle (Figure 5, right-hand panel lanes D and E) show that, although the resolution of products of digestion is limited near the top of each gel lane, there is a clear pattern of protection extending from the gel origin to position 700. This portion of DNA contains binding sites at positions 688 and 710. Since the protection here is very strong when compared with that at other CpG sites in the same gel lanes and it resembles the pattern for free DNA plus echinomycin (lane B in Figure 5, right-hand panel) we suggest that these binding sites lie beyond the edge of the core particle proper, as a result of which they have a greater affinity for the antibiotic. Such an effect has previously been reported for daunomycin [17] which has been shown to have a much greater affinity for internucleosomal DNA than for DNA bound to the protein core. This leaves only three potential binding sites for echinomycin in the DNA bent around the histone octamer, in the vicinity of positions 778, 810 and 828. It then appears that echinomycin molecules must be protecting both outward and inward facing sites on the DNA, though in the absence of a single clear periodicity in the native core particle [4] it is impossible to draw any absolute conclusions save that some sort of rotational change has probably occurred in the presence of the antibiotic.

A Fourier analysis of the data is more encouraging: it provides (Table 1) objective evidence for a regular periodic variation, with maximum amplitude at a period of 10.87 bp. This represents the highest value observed with core particles in the presence of any antibiotic [1,2] (other than the spurious value of 12.2 calculated above for distamycin) and is significantly larger than the value of 10.7 calculated for one set of peaks in "core 5" [4] although less than the value of 11.0 calculated for the other set of "split" peaks. Using these numbers a rough calculation of the number of bound echinomycin molecules was performed, based on the 10.87 period and the control 10.7 value, as described elsewhere [1]. The relative positions of maxima in Figure 6(b) differ by 109 bonds over 10 periods, compared with 107 bonds for one set of "split" peaks in the native core [4]. Thus, echinomycin has unwound the DNA helix by about the same angle as two base pairs, or $2 \times 36^{\circ} = 72^{\circ}$. This yields a value of $72^{\circ}/48^{\circ} = 1.5$ molecules bound per nucleosome core on average, probably best regarded as a lower limit.

DISCUSSION

In the first paper on this subject [1] it was shown that both echinomycin and distamycin A induce the tyrT DNA fragment to rotate with respect to the

histone octamer by about half a turn. We have now presented experimental results using two different pieces of DNA from a eukaryotic source which reveal that they too can be induced to rotate on the surface of the histone octamer in the presence of antibiotics. As previously suggested [1] it seems likely that the induced change in rotational setting occurs as a result of binding of the antibiotics to their preferred nucleotide sequences, presumably serving to optimise non-bonded contacts between the ligand and the polynucleotide backbone [1,2]. Although this explanation is generally consistent with all our data there are some differences in the behaviour of the various DNA fragments which need to be explained. Since the results obtained with the 135-mer core particles are generally easier to interpret than those obtained with the 189-mer cores they will be considered first.

In practically all respects, particles reconstituted using the 135-mer DNA fragment behave in a similar fashion to particles containing tyrT DNA [1]. Echinomycin and distamycin are both able to change the rotational orientation of the DNA with respect to the histone octamer, and the effect of distamycin is probably the clearest and most unambiguous observed to date (Figures 3 and 4(a)), witness also the very high amplitude of the Fourier periodicity calculated in Table 1. It is clear that the results cannot be explained by suggesting that the observed cleavage pattern is the result of a simple mixture of unmodified core particles together with displaced, free DNA. Although certain new bands asterisked in Figures 3 and 5 are evident in digests of the free 135-mer DNA in the presence (or absence) of antibiotic, other bands are only seen in the core-antibiotic cleavage products and can barely be detected, or not at all, in free DNA complexed with the antibiotics.

The situation is perhaps more complicated with echinomycin. It was tentatively suggested in [1] that gross rotation of nucleosome core DNA might at least partially be caused by the helix-unwinding effect of echinomycin upon binding to DNA, but an unwinding effect per se is not sufficient to cause rotation because the well-characterised intercalators nogalamycin and actinomycin fail to elicit the same response [2]. Moreover, the periodicity calculated in the Fourier analysis (Table 1) is very close to the value calculated for the core DNA alone in the absence of any antibiotic [4], which suggests that the total perturbation of the winding of the fundamentally B-form helix must be very small - equivalent to binding of only one echinomycin molecule to the same length of unconstrained DNA - yet the whole helix appears to have rotated with respect to the protein core.

The effects of antibiotic binding on nucleosome core particles containing the longer 189-mer DNA are not easy to compare with those from cores containing the other two fragments because the reconstituted nucleosomes exhibit no single regular periodicity of cleavage,indicative of a poorly ordered rotational setting [4]. Despite this, both echinomycin and distamycin have a definite influence on the pattern of fragments produced by DNAase I digestion. Since only about 145 bp can be accommodated within the core particle structure itself [3,4,8,12] the extra naked DNA presents another complicating factor. There are several antibiotic-protected binding sites in this naked portion of the DNA fragment (Figures 3 and 5, right-hand panels). It has been reported that anthracycline antibiotics such as daunomycin prefer to bind to internucleosomal DNA regions over the polynucleotide wrapped around nucleosomes [17,18], and we suspect that a similar phenomenon applies with both distamycin and echinomycin.

A careful inspection of the results presented here and in related papers [1,2] suggests that some of the new bands which appear in the presence of antibiotics may be accounted for by enhanced nuclease cutting at nucleotide sequences flanking certain strong ligand-binding sites [7,10]. The distribution of the available binding sites further complicates the distinction between effects of rotation, protection, new DNAase I cutting sites and antibiotic-induced enhancement of cleavage. However, in the case of echinomycin binding to the 189-mer core particle we can study a reasonably long stretch of DNA extending between bonds 780 and 808 approximately (Figure 6) where no specific CpG binding sites are present. The new electrophoretic bands occurring in this region, marked with asterisks in Figure 5, can only be attributed to the rotation of DNA and cannot be explained either in terms of a mixture of unmodified cores together with displaced DNA or by enhancements induced by antibiotic binding. As regards the number and distribution of available binding sites we note that the small differences between core particles containing tyrT DNA or the 135-mer fragment can probably be attributed to the clustering of potential antibiotic binding sites in the former DNA [1-3,7,10] whereas in the latter they are generally isolated from each other (see Figures 4 and 6).

Wu et al. [19] have reported electric dichroism and ultracentrifugation experiments which reveal that binding of ethidium produces structural distortion (unfolding) of nucleosome core particles. It is very likely that their reported changes in particle shape and our observations of altered

nuclease cleavage patterns can occur together and, indeed, may share a common origin. Both phenomena point to the occurrence of conformational changes associated with the binding of small ligands, without gross detachment of DNA from the histone octamer. An advantage of nuclease accessibility studies is that, as in the case of distamycin binding to the 189-mer core particles, we can detect changes in the rotational orientation of DNA even when some of the DNA does appear to become detached from the protein core.

In summary, the present results provide evidence that the rotation of DNA on the surface of nucleosome core particles induced by several drugs is not a peculiarity of the DNA fragment employed, but a general phenomenon. Its occurrence is in no way incompatible with the theoretical analysis of DNA motions in the core particle [20]. Moreover, the observation that several new bands appear in nuclease digestion patterns distributed mid-way between control core bands, even in regions where no antibiotic can bind, points strongly to the conclusion that these bands cannot result from enhanced cutting at sequences flanking the binding sites but reflect a new rotational orientation of DNA about the histone octamer.

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