
Echinomycin and distamycin induce rotation of nucleosome core DNA

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Received 17 July 1986; Accepted 18 August 1986

ABSTRACT

When nucleosome cores reconstituted from chicken erythrocyte histones and a 160 bp DNA molecule are exposed to echinomycin, a *bis*-intercalating antitumour antibiotic, the DNA appears to rotate with respect to the histone octamer by about half a turn. New bands appear in patterns of DNAase I digestion at positions approximately mid-way between those characteristic of control core samples, while the control pattern is largely suppressed. Similar (but not identical) changes are produced when nucleosome cores are exposed to distamycin, a non-intercalating DNA-binding antibiotic. The effects of both ligands can be explained in terms of a change in rotational orientation of the core DNA, so as to place antibiotic binding sites on the inward-facing (concave) surface of the DNA supercoil. Presumably this serves to optimise non-bonded contacts with the polynucleotide backbone. These results establish that the positioning of DNA about the histone octamer is not absolutely determined by its nucleotide sequence, but may be modified by the binding of such relatively small molecules as antibiotics.

INTRODUCTION

Echinomycin and distamycin are both antibiotics which kill tumour cells in culture and display significant antiviral activity, although they are generally too toxic for clinical use [1,2]. Echinomycin (Figure 1) binds to DNA by a mechanism involving simultaneous intercalation of its two quinoxaline chromophores. "Footprinting" experiments have revealed that it recognises the nucleotide sequence CpG [3,4]. Distamycin (Figure 1), on the other hand, binds to AT-rich regions in the minor groove without intercalating between base pairs [5-7]. From solution studies, it has been reported that both antibiotics are able to induce conformational changes in regions of the double helix flanking their binding sites. These are seen as regions which are cut better by DNAase I in the presence of the antibiotics. The effects are not restricted to any particular category of base-sequence; for example, they are produced in runs of A and T by echinomycin, and in GC-rich sequences by distamycin [3,7].

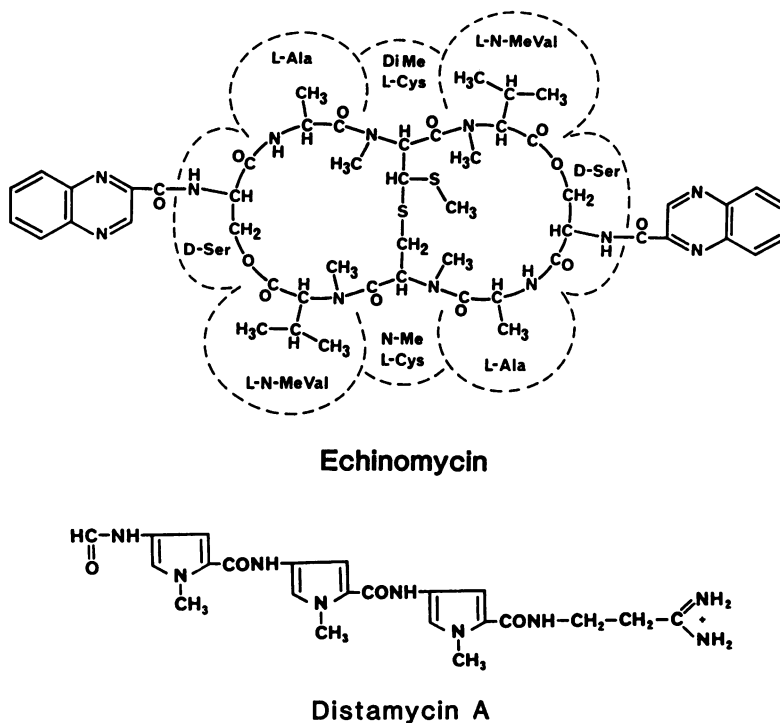


Figure 1. Chemical structures of echinomycin [14] and distamycin A [5].

Additional information regarding the binding specificity of these antibiotics comes from the crystal structures of echinomycin, triostin and netropsin (which is structurally related to distamycin), all of which have been complexed with short DNA fragments. The specificity of echinomycin for the sequence CpG is probably explained by hydrogen-bonded interaction between the amino groups of guanine nucleotides and the carbonyl groups of alanine residues in the depsipeptide ring [8-10]. On the other hand, the preference of netropsin for binding to AT residues seems to be determined primarily by van der Waals contacts between pyrrole rings of the antibiotic and various surfaces on the DNA. Hydrogen bonds from netropsin amides serve only to position the drug molecule more precisely within its preferred binding site [11].

Clearly a wealth of information has been gained from solution and crystallographic studies concerning the binding of echinomycin and distamycin to naked DNA. In the cell, however, the DNA is complexed with a collection of

histones and other nuclear proteins. Can antibiotics bind to this DNA, and what effects might they have on its structure?

To explore this question we have studied the interaction of echinomycin and distamycin with nucleosome core particles containing a 160 bp DNA molecule of defined sequence. Upon addition of either echinomycin or distamycin to these core particles, we find that the DNA rotates with respect to the surface of the protein by roughly half a turn. Binding sites for either antibiotic which were originally positioned along the outer surface of the DNA supercoil have been turned inward, presumably to improve non-bonded contacts with the DNA sugar-phosphate backbone.

EXPERIMENTAL PLAN

We constructed nucleosome core particles of defined sequence by the method of salt exchange [12,13], using a sample of freshly-prepared chicken nucleosome cores and a 160-base-pair DNA molecule identical in sequence to that used previously to study the binding of echinomycin and distamycin to naked DNA [3,7]. Next, we subjected these core particles to digestion by DNAase I in the absence and presence of varying concentrations of antibiotics. The products of digestion were then isolated from the protein and applied to an 8% denaturing polyacrylamide gel in order to visualise them at single-bond resolution.

A full structural analysis of these defined-sequence core particles has already been reported, in relation to the problem of nucleosome positioning [13]. It seems that the positioning of DNA about the histone octamer depends primarily on the sequence-dependent anisotropy of DNA bending. Certain base sequences, typically runs of A and T, prefer to sit with their minor grooves facing inward towards the protein, while others, often runs of G and C, are preferentially located with the minor groove facing out and away from the protein. Our results reveal that these sequence-dependent preferences for DNA bending are not absolute, but may be modified by the interaction of the DNA with simple antibiotics such as echinomycin and distamycin.

MATERIALS AND METHODS

Antibiotic solutions

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. Concentrations of echinomycin were determined spectrophotometrically from the absorbance at 325 nm ($\epsilon_{325} = 11,500 \text{ M}^{-1} \text{ cm}^{-1}$)

[14]. Because of its low solubility in water (5 μ M), stock solutions were prepared in a methanol-buffer mixture (30/70, v/v) as in previous studies [3], so that the final concentration of methanol present in the reaction mixture did not exceed 15%. The aqueous phase contained 10mM Tris-HCl, pH7.5 and 10mM NaCl. Solutions of distamycin were prepared freshly by direct weighing and were dissolved in the same buffer as used for echinomycin, without the methanol.

Preparation and digestion of the core complex

The nucleosome core particle was prepared as described by Drew and Travers [13]. Approximately 500ng of tyrT DNA were cut out of plasmid pKMA-98 by the use of EcoRI and AvaI, treated with reverse transcriptase and [α -³²P]dATP or dCTP so as to label selectively one of the two recessed 3'-ends left by the restriction digest, then isolated by excision from a 6% non-denaturing polyacrylamide gel. This purified material was then incubated with a 100-fold molar excess of nucleosome core particles from chicken erythrocytes (a gift from Dr D. Rhodes) in a 20 μ l solution containing 20mM Tris, pH7.8, 700mM NaCl, 0.2mM EDTA and 0.2mM PMSF for 20 minutes at 37 $^{\circ}$ C. Within the space of a few minutes, all of the labelled tyrT DNA exchanges with a small fraction of the unlabelled chicken core DNA molecules [13]. In order to "freeze" this equilibrium, the salt concentration was lowered from 700 to 100mM NaCl, by the stepwise addition of 10mM Tris in 5 μ l aliquots (once every 10 minutes, 20 $^{\circ}$ C).

Digestion of the reconstituted material with DNAase I was performed in parallel with the digestion of a free DNA control, which had been taken through all of the steps of reconstitution without having been exposed to nucleosomes. In a typical experiment, 35 μ l of core complex (or free DNA) were incubated with an equal volume of an antibiotic (or water) for 30 minutes, then adjusted to 1mM MgCl₂ and subjected to DNAase I digestion (10 minutes, 37 $^{\circ}$ C) at an enzyme concentration of 5.0 units/ml for core, or 1.0 units/ml for free. The reaction was stopped by adjustment to 2mM EDTA and the histone octamer was removed by extensive digestion with proteinase K (0.5 mg/ml) for 30 minutes at 37 $^{\circ}$ C in the presence of 1% SDS. The reaction mixture was then extracted twice with phenol/chloroform, twice with ether, adjusted to 0.3M Na acetate, and the DNA recovered by ethanol precipitation. Products of digestion were fractionated on 8% denaturing polyacrylamide gels containing 7M urea. Bands in the tyrT digestion pattern were assigned by comparing the pattern in control lanes with that previously determined [13,15].

Densitometry

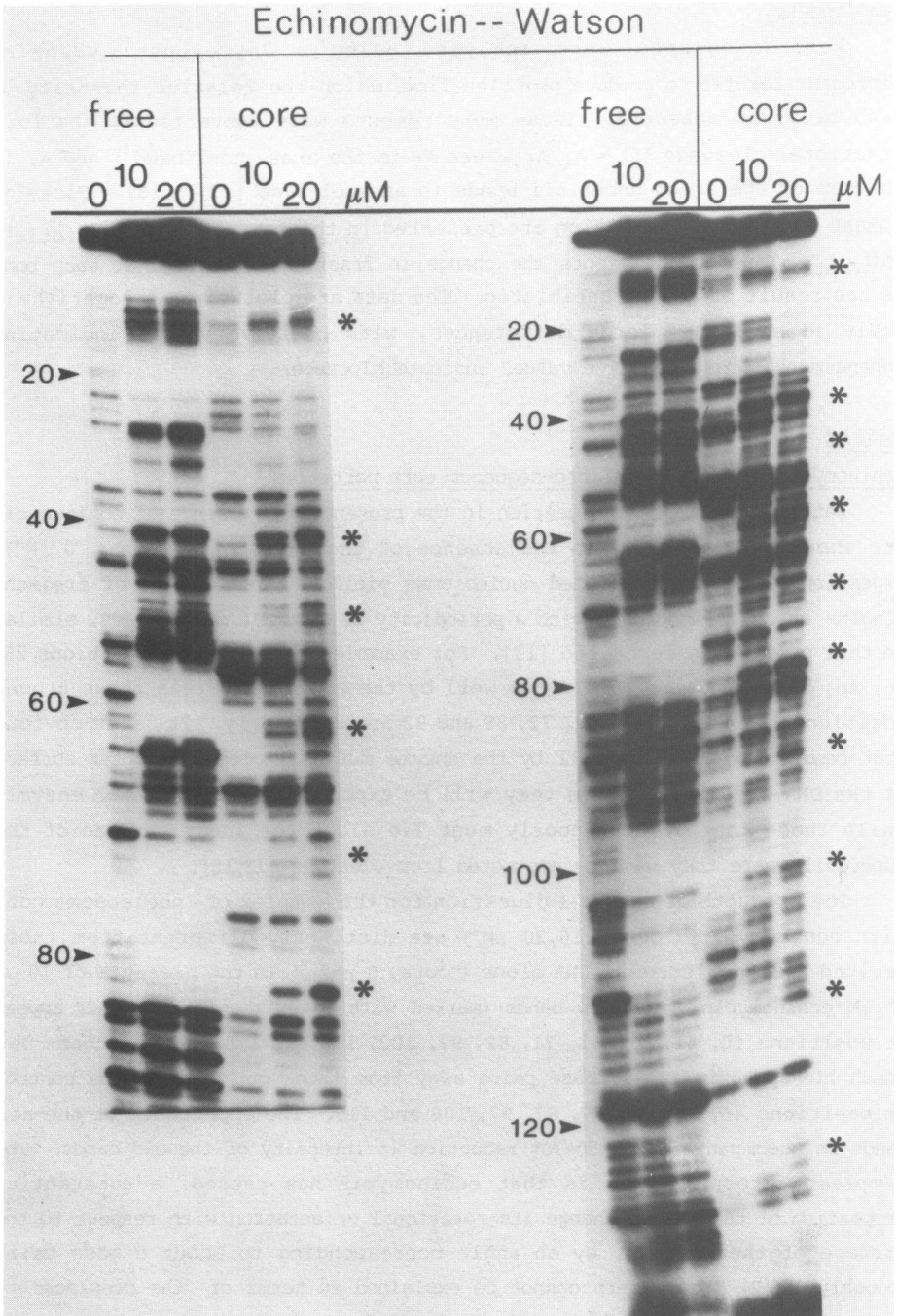
Autoradiographs were analysed using a Joyce-Loebl scanning microdensitometer to produce profiles from which the relative intensity of each band was measured. These measurements were converted to the form fractional cleavage (f) = A_i/A_t where A_i is the area under band i and A_t is the sum of the areas under all bands in any gel lane [3,15,16]. Plots of DNAase I cleavage inhibition are presented in the form of $\ln(f_{\text{antibiotic}}) - \ln(f_{\text{control}})$ which represents the change in fractional cleavage at each bond as the result of adding antibiotic. The data are plotted on a logarithmic scale to encompass large differences, with positive values indicating enhancement, while negative values indicate blockage.

RESULTS

Echinomycin binding to defined-sequence core particles

Patterns of DNAase I digestion in the presence and absence of echinomycin are shown in Figure 2. In the absence of the antibiotic ("core, 0 μM "), digestion of the reconstituted nucleosomes yields a distribution of fragment lengths which is modulated with a periodicity of about 10 nucleotides, similar to that previously described [13]. For example, bonds around positions 25, 36, 46, 56, 67, 77 and 87 are cut well by the enzyme, whereas those around positions 20, 31, 41, 51, 62, 72, 82 and 92 are cut poorly. It seems obvious that bonds which are cut well by the enzyme must lie along the outer surface of the DNA supercoil, where they will be exposed to attack by the enzyme, while those that are cut poorly must lie along the inner surface of the supercoil where they will be protected from cleavage [13,16].

The products of DNAase I digestion for the complex of nucleosome core with echinomycin ("core, 10,20 μM ") are distinctly different from those derived from nucleosomal DNA alone ("core, 0 μM "). In the presence of 10 or 20 μM echinomycin, many new bands (marked with asterisks in Figure 2) appear at positions 10, 41, 51, 61, 71, 82, 92, 103, 114 and 125. All of these new bands lie approximately 5 base pairs away from those cut well in the control at positions 46, 56, 67, 77, 87, 97, 108 and 119. The appearance of the new bands is accompanied by a 50-70% reduction in intensity of the old bands. The simplest interpretation is that echinomycin has caused a substantial proportion of the DNA to change its rotational orientation with respect to the surface of the protein, by an angle corresponding to about 5 base pairs (roughly 180°). The pattern cannot be explained in terms of the displacement of a certain fraction of the labelled DNA from the histone octamer, even



though some of the new bands (such as 10, 41, 92 and 125) are characteristic of free DNA in the presence of echinomycin (Figure 2). Other bands, notably at positions 61, 71 and 82 are totally new in the core digest and virtually absent in free DNA complexed with echinomycin: several more (51, 103, 114) are obviously strongly enhanced in the nucleosome core but suppressed in free DNA. Moreover, the evident periodicity in the occurrence of the new bands, once every 10-11 nucleotides, speaks for a firm constraint not enjoyed by the DNA molecule alone.

We conclude that rotation of the entire helix on the surface of the nucleosome must have occurred. Regions of the DNA which originally faced inward towards the histone octamer now face outward, away from the protein, while conversely those regions which originally faced outward now face in. No more than 50-70% of the DNA molecules could have undergone this change in rotation because the original pattern of digestion is still apparent, although reduced in intensity. The observed changes in the pattern of digestion were found to remain constant between 10 and 30 μM antibiotic; at concentrations above 30 μM the digestion pattern of the echinomycin-core complex began to resemble that obtained for the complex of echinomycin with DNA free in solution.

In order to attempt a more quantitative assessment of these data, the relative intensities of all individual bands in each gel lane were measured by densitometry and converted into a plot of differential cleavage, as shown in Figure 3. This plot depicts the probability ratio for digestion at each individual bond of DNA bound to the nucleosome core in the presence and absence of echinomycin. All the points are plotted on a logarithmic scale; positive values indicate enhanced cleavage by the enzyme, while negative values indicate protection from cleavage. The new bands that appear in the presence of echinomycin (asterisks in Figure 2) can now be seen as distinct maxima in the difference plot at positions 10, 20, 41, 50, 61, 71, 82, 92, 103, 114 and 125 on the upper Watson strand. On the lower Crick strand,

Figure 2. DNAase I footprinting of echinomycin bound to the free *tyrT* DNA fragment or to the reconstituted nucleosome core. Two gels derived from the same set of digestion mixtures are shown: that on the left was run for a long time so as to improve resolution of the longer fragments (bands 10-90). In this experiment the transcribing "Watson" strand (upper sequence, 5' to 3' left-to-right in Figure 3) was labelled at its 3' end. Each set of three tracks represents a control (no antibiotic) together with samples containing 10 or 20 μM echinomycin as shown at the top of each lane. Numbers at left refer to the numbering scheme shown in Figure 3. Asterisks indicate the new bands which appear in digests of core particles treated with echinomycin.

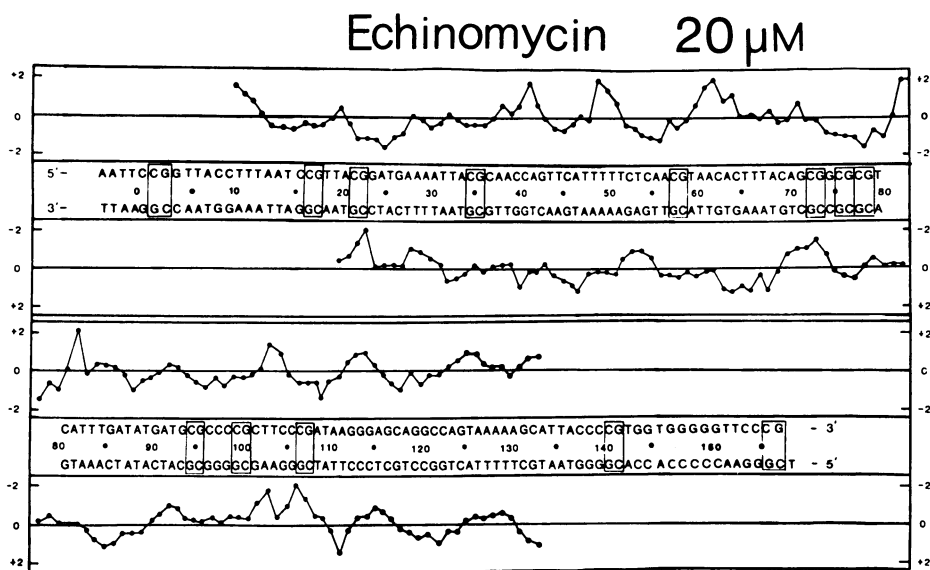


Figure 3. A plot of differential cleavage representing the effect of echinomycin (20 μ M) on susceptibility of nucleosome core particles to attack by DNAase I. The upper "Watson" strand reads 5' to 3' left-to-right whereas the lower "Crick" strand reads 5' to 3' right-to-left. Vertical scales on both sides are 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 total digestion. Positive values indicate enhancement, negative values blockage.

maxima are found around positions 40, 46, 66, 77, 86, 111 and 122. The two sets of maxima are displaced with respect to each other by a mean stagger of 3-4 bonds towards the 3'-end of each strand. For example, maxima occur at positions 41 (Watson) and 40 (Crick), 50 and 46, 71 and 66, 82 and 77, 92 and 86, 114 and 111, 125 and 122. This cross-strand stagger occurs because DNAase I cuts at phosphates which lie in close proximity across the double-helical minor groove, but are separated along the helix by a distance of 3-4 base pairs [3,13,15-17].

In order to test our interpretation of the digestion data, all of the values from the difference plot of Figure 3 were subjected to Fourier analysis. If 50-70% of the DNA molecules have rotated by one-half turn, then a difference function of "core, 20 μ M antibiotic" minus "core, no antibiotic" should show a strong periodicity of about 10 bp, the maxima of which should be equivalent to the maxima apparent in Figure 3. The results of this Fourier analysis are summarized in Table 1. There is indeed a strong, regular

Table 1. Fourier Analysis of the Differences in Digestion Between "Core, 20 μ M Antibiotic" versus "Core, No Antibiotic".

<u>Antibiotic</u>	<u>Amplitude</u>	<u>Period(bp)</u>	<u>Positions of maxima, Watson strand</u>
Echinomycin	25.4	10.64	18.2,28.8,39.5,50.1, 60.8,71.4,82.0,92.7, 103.3,114.0,124.6
Distamycin	22.4	10.42	19.8,30.2,40.6,51.1, 61.5,71.9,82.3,92.7, 103.2,113.6,124.0

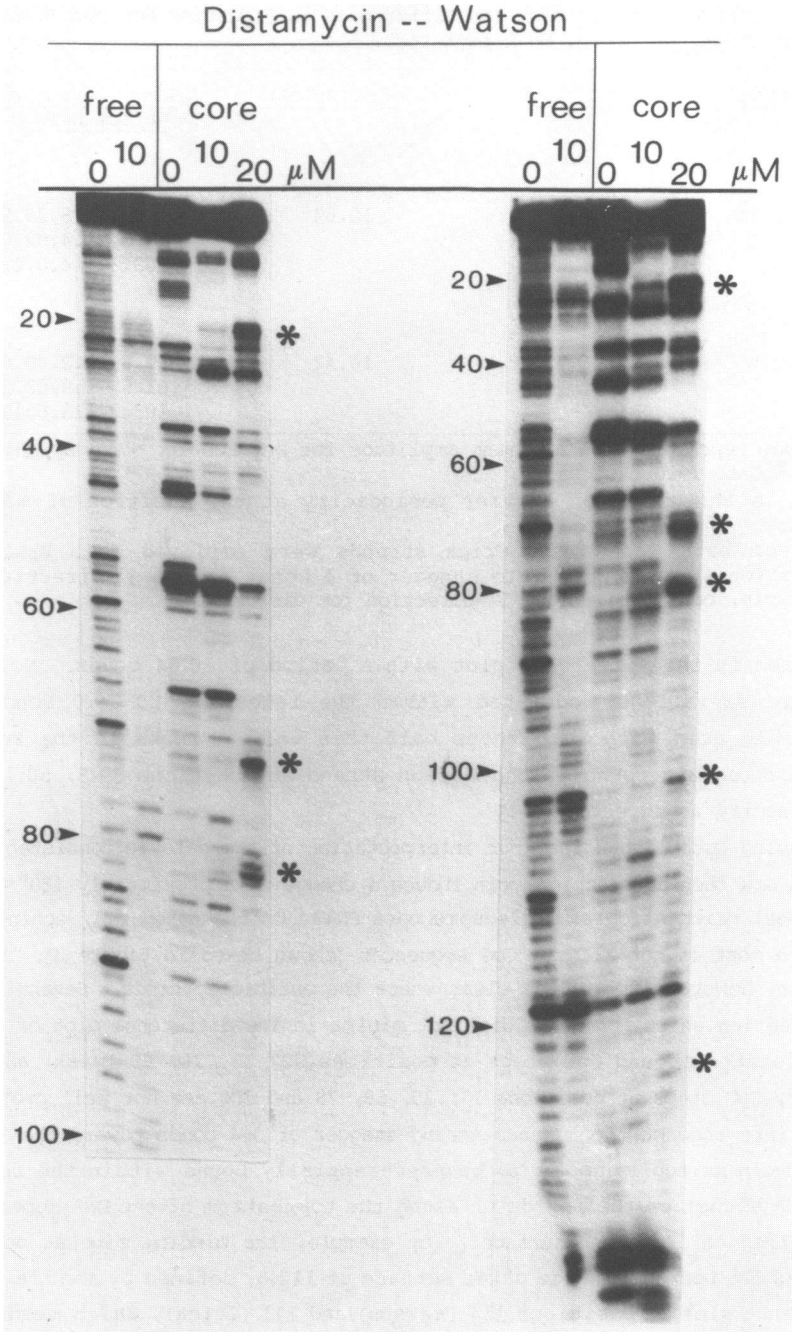
Amplitude represents the maximum amplitude for any Fourier wave spanning the region 5.0 to 19.0 base pairs.

Period is the value of Fourier periodicity at the position of maximum amplitude.

Data from both Watson and Crick strands were employed as a basis for calculation at an appropriate stagger of 4 bonds in the 3'-direction for echinomycin, or 1 bond in the 3'-direction for distamycin.

variation in the difference plot with a period of 10.64 bonds. No other periodic variations modulated within the range 5.0 to 19.0 bonds are detectable even with amplitudes half this value. Peaks in the Fourier distribution are found on the Watson strand at positions 39.5, 50.1, 60.8 etc., exactly as expected.

Having established that our interpretation of the data is consistent with theory, how then does echinomycin induce a change of approximately 180 $^{\circ}$ in the rotational orientation of nucleosome core DNA? On the naked DNA, echinomycin binds to most if not all the CpG sequences (shown boxed in Figure 3). Within the core, however, it is less clear where the antibiotic binds. Several sites of protection which appear as distinct minima in the difference plot of Figure 3 are located around CpG steps at positions 22, 73, 76, 95 (weak) and 107. However, CpG steps at positions 17, 35, 58, 78 and 100 are not well protected. Taking into account the cross-strand stagger of 3-4 bonds toward the 3'-end, echinomycin molecules appear to be preferentially bound within the rotated core DNA either on the inside or along the top/bottom of the DNA supercoil, rather than on its outer surface. For example, the binding site at position 107 lies 5.5 bonds from the outer surface at 112.5, defined by maxima in the difference plot at positions 114 (Watson) and 111 (Crick), which means that the antibiotic lies on the inside of the supercoil. Similar arguments hold for the binding sites at positions 22, 73, 76 and 95. On the other hand, the



CpG sequence at position 58 lies 10 bonds away from the outer surface at 48.0, as defined by maxima at positions 50 (Watson) and 46 (Crick). It is therefore located on the outer surface of the DNA, yet apparently does not bind the antibiotic. Similarly, the CpG steps at positions 17, 35, 78 and 100 lie predominantly along the outer surface of the DNA supercoil. It therefore appears that echinomycin induces a 5 base-pair rotation of the DNA helix by binding to certain sites containing CpG that are exposed on the outside of the DNA in the antibiotic-free core particle, and turning them inwards to face the histone octamer.

A very rough estimate of the number of echinomycin molecules bound per DNA molecule can be made by comparing the known unwinding angle of the antibiotic (48° , [14,18]) with the observed change in helical periodicity of the DNA in the echinomycin-nucleosome core complex. The relative positions of maxima on the Watson strand differ by $(125-19) = 106$ bonds over 10 periods, as compared with 103 bonds over the same ten periods in the absence of the antibiotic [13]. Thus, echinomycin appears to have unwound the DNA by no more than three base pairs, or $3 \times 36^\circ = 108^\circ$. This indicates that there can be only about $108^\circ/48^\circ = 2.25$ antibiotic molecules bound per nucleosome core particle, and they may be distributed statistically over many sites.

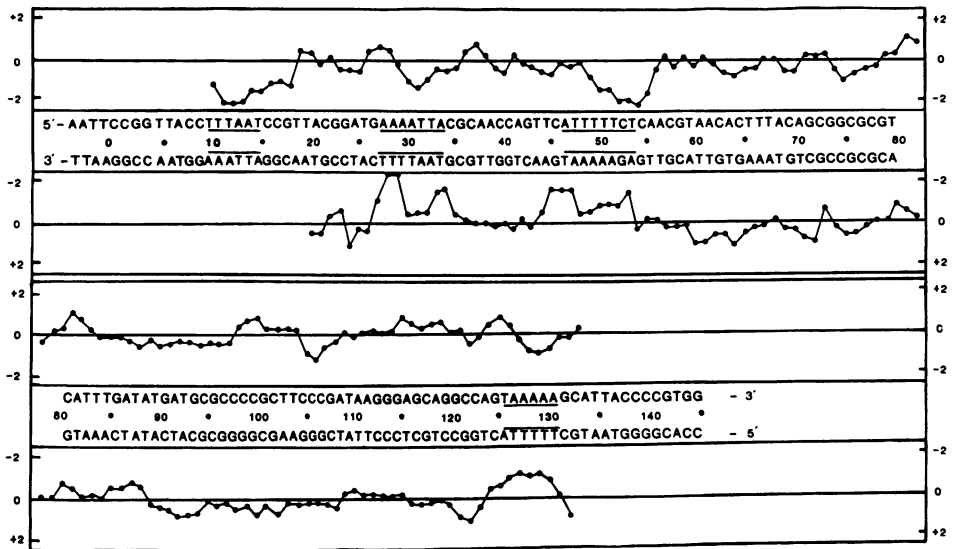
The results show that echinomycin, a bifunctional intercalator specific for CpG, induces large changes in the rotational orientation of nucleosome core DNA. Can distamycin, which binds in the minor groove at AT-rich regions, also affect the conformation of DNA bound to the protein?

Distamycin binding to defined-sequence core particles

Patterns of DNAase I digestion for the same nucleosome core particles in the presence of 10 and 20 μM distamycin are shown in Figure 4. In the presence of 10 μM distamycin ("core, 10 μM "), slight changes can be seen but the pattern closely resembles that of the unperturbed nucleosome core DNA ("core, 0 μM "). Upon addition of 20 μM antibiotic, the changes become more pronounced. New bands appear at positions 20, 71, 81, 103 and 123 (marked with asterisks in Figure 4), all of which lie about 5 bonds away from those previously cut well at positions 25, 36, 46, 56, 67, 77, 87, 108 and 119 in the native core DNA. Moreover the old set of bands is almost completely suppressed. Again it appears that in the presence of distamycin, about 90% of the DNA molecules have rotated by one-half turn in the same way as for

Figure 4. DNAase I footprinting of distamycin bound to free tyrT DNA or to the reconstituted nucleosome core. Presentation and details as described in the legend to Figure 2.

Distamycin 10 μ M



Distamycin 20 μ M

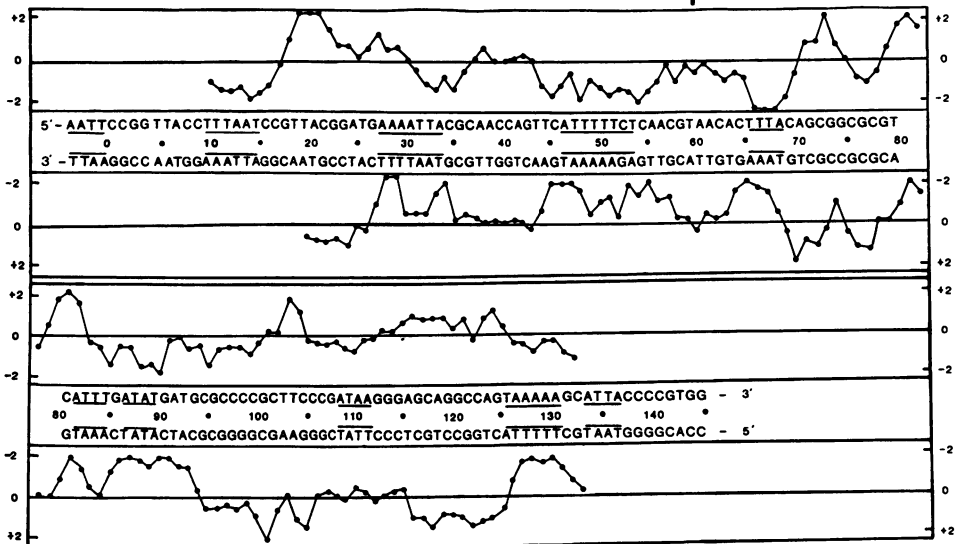


Figure 5. Plots of differential cleavage representing the effects of distamycin, 10 μ M (a) or 20 μ M (b), on susceptibility of nucleosome core particles to digestion by DNAase I. Details as described in the legend to Figure 3.

echinomycin described above. Those regions of DNA which were originally hidden inside the supercoil become exposed for cleavage, while those that were outside become protected from cleavage. As before, it is not correct to interpret the antibiotic-induced pattern of digestion in terms of a mixture of unmodified cores together with displaced, free DNA. For example, in the left-hand part of Figure 4, bands appear at positions 76-79 for distamycin bound to free DNA ("free, 10 μM "), but at positions 71 and 81 for distamycin bound to the nucleosome core ("core, 20 μM "). Numerous examples of this sort can be found. By careful densitometric analysis of each gel lane, we have converted the data into a differential cleavage plot depicting the difference in enzymatic cleavage in the presence and absence of 10 and 20 μM distamycin, as shown in Figures 5(a) and 5(b) respectively. The new bands which appear in the "distamycin, core" lanes are seen as maxima in these plots. In Figure 5(b) distinct maxima are found at positions 20, 72, 81, 103 and 124 on the Watson strand. The corresponding maxima on the Crick strand are staggered by 2-4 bonds towards the 3'-end at positions 70, 77, 100 and 120.

Again, in order to test our interpretation objectively, we subjected all values from the difference plot of Figure 5b to Fourier analysis. If 90% of the DNA molecules have rotated by one-half turn, then the difference between "core, 20 μM antibiotic" and "core, no antibiotic" should show a strong, regular variation with a periodicity of about 10 bp, the maxima of which should coincide with the maxima in Figure 5b. The results are presented in Table 1. In the case of distamycin, the maximal amplitude occurring in the region between 5.0 and 19.0 is found at 10.42 bonds, slightly less than the 10.64 for echinomycin. (This is to be expected, since distamycin does not unwind the DNA helix). The positions of maxima on the Watson strand lie at 19.8, 30.2, 40.6, etc., as expected.

What can we deduce about the disposition of sites bound by distamycin at low and high concentrations? In the presence of 10 μM distamycin, antibiotic-induced blockages, evident as minima in Figure 5(a), are located at sequences TTTAAT, AAAATTA, ATTTTCT and TAAAAA around positions 13, 30, 50 and 130 respectively. These AT-rich sequences are located on the inside of the DNA supercoil in the native core particle [13]. Therefore, at low concentrations, distamycin binds preferentially to AT-rich regions which lie along the inside of core DNA. These regions are marginally protected from enzyme digestion along the top or bottom of the DNA supercoil, where DNAase I has moderate access. At 20 μM , the antibiotic binds at many additional sites. As shown in Figure 5(b), short regions rich in A and T such as those centred around

positions 67, 83, 89 and 110 also become protected. These short runs of A plus T lie along the outer surface of the DNA in the native core particle, as judged by their sensitivity to DNAase I digestion [13]. It therefore appears that at high drug concentration, distamycin can bind to runs of A plus T on both the inside and outside of core DNA. We will consider later how this may bring about the observed change in rotation.

It is not possible to estimate the number of distamycin molecules bound per DNA molecule in these experiments because distamycin does not substantially alter the periodicity of the double helix in either free or core DNA. However, since nearly every run of 4-6 A/T base pairs is protected from cleavage at a concentration of 20 μM , it seems likely that at this concentration the nucleosome core can accommodate approximately 5-10 antibiotic molecules.

DISCUSSION

It has previously been shown that both echinomycin and distamycin can influence the conformation of DNA at sequences flanking their binding sites, apparently by different mechanisms [3,7]. Echinomycin unwinds the helix by 48° so as to open the minor groove nearby: when the flanking sequences are of the type (dA).(dT), which have been observed to adopt a narrow minor groove in the absence of antibiotic [19,20], this opening of the groove to an intermediate form seems to enable DNAase I to cut more rapidly [3,21]. By way of contrast, distamycin lies deep within a narrow minor groove and makes van der Waals contacts with sugar-phosphate chains on either side [11]: for sequences of the type (dG).(dC) which have been seen to adopt a wide minor groove in the absence of antibiotic [22,23], the nearby presence of distamycin is thought to reduce the spacing between phosphate chains to an intermediate value, and similarly encourage DNAase I to cut more rapidly [7,21]. Our present results reveal that, on binding to nucleosome core DNA, the two antibiotics again influence the DNA conformation. This becomes apparent as a change in the rotational orientation of the double helix, apparently without its removal from the protein surface. We may now consider by what mechanism the antibiotics bring about such changes.

In the case of distamycin, the change in rotational positioning is only seen at high concentrations of antibiotic under circumstances where the drug binds to both the inside and the outside of the DNA supercoil. The preference of the ligand for binding to sites that lie on the inside of the supercoil can be deduced from its distribution at low concentration; presumably the narrow

dimensions of the minor groove on the inside of a tightly-bent DNA molecule [13] improve non-bonded interactions between the antibiotic and the DNA sugar-phosphate backbones. This is in accord with the crystal structure of netropsin (which is structurally related to distamycin) bound to the sequence AATT [11], where the width of the minor groove is just 10Å as compared with a value of 12-13Å elsewhere in the DNA molecule and 15-17Å in other crystalline sequences. It seems plausible therefore that high concentrations of distamycin could favour a change in rotational orientation by binding first to AT-rich regions on the outside of the supercoil, and then turning them inwards so as to improve non-bonded contacts on either side. Such a rotation might also require a translation of the DNA along the surface of the histone octamer, such that the essential contacts of the histones with DNA phosphates could be maintained. The required translation would be 5 bp, or 5 bp plus any integral number of double-helical turns (e.g. 15, 26, 36).

The situation is more complicated with echinomycin, for which the binding sites of the antibiotic on core DNA are not entirely clear. We may forward a number of observations. Firstly, it is very likely that the gross change in helical orientation is at least partially caused by the unwinding effect of echinomycin upon binding to DNA. The distortion caused by the antibiotic might be so great that very few molecules can be tolerated by each nucleosome core particle. The DNA evidently detaches from the histone octamer when more are bound. Our measurements, indeed, suggest that on average the core particle can only accommodate 2.25 echinomycin molecules per duplex. But the results of Drew & Travers ([13]) establish that there are at least 5 CpG sequences located on the exposed outside surface of the supercoil, and thus presumably susceptible to binding by echinomycin. If the affinity of the antibiotic for each of these sites is approximately the same then each site is likely to be only 45% occupied upon addition of the antibiotic. Consequently the protection against DNAase I cleavage might be relatively weak, as observed. But why should the entire helix appear to rotate with respect to the histone octamer? As in the case of distamycin, we favour the concept of an inward movement of binding sites so as to maximise non-bonded interaction(s) between the antibiotic and the DNA sugar-phosphate backbones. When the DNA helix is bent to fit around a histone octamer its grooves become wide on the outer surface but narrow along the inward-facing surface of the supercoil [24]. Further widening along the outer surface, due to intercalation-induced extension and unwinding, is unlikely to facilitate favourable interaction with the peptide moiety of an antibiotic such as echinomycin. Such interactions

are clearly evident in the crystal structure of echinomycin bound to the sequence d(CGTACG) where a major proportion of the stabilization of the antibiotic-DNA complex arises from non-bonded interactions between the atoms of the octapeptide ring and the sugar-phosphate backbone [10]. Accordingly a half-turn change in the rotational orientation of the entire helix so as to bring the bound CpG sequences to an inward-facing position may be strongly favoured.

In summary, the relevance of this work to biology is threefold. Firstly, we have shown that the rotational positioning of DNA about the histone octamer is not absolute, but may be modified by the binding of simple antibiotics. Secondly, we have learned more about the possible mechanisms by which these antibiotics affect the functioning of the cell nucleus. Thirdly, the present data provide a model system in which to study the transmission of a conformational change in DNA over long distances. Some of the earliest analogue computers were built to occupy an entire room, and employed long metal rods to transmit information from one operating station to another. It will be interesting to see whether an analogous mechanism is at work in chromatin, perhaps even to alter the specific exposure or "sidedness" of the DNA over several nucleosomes.

ACKNOWLEDGMENTS

We thank Drs K.R. Fox and A. Klug for help and encouragement, and Mark Freeman for able technical assistance. This work was supported by grants from the Cancer Research Campaign, the Royal Society and the Medical Research Council.

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