Molecular modelling study of changes induced by netropsin binding to nucleosome core particles

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ABSTRACT

It is well known that certain sequence-dependent moduladors in structure appear to determine the rotational positioning of DNA on the nucleosome core particle. That preference is rather weak and could be modified by some ligands as netropsin, a minor-groove binding antibiotic. We have undertaken a molecular modelling approach to calculate the relative energy of interaction between a DNA molecule and the protein core particle. The histones particle is considered as a distribution of positive charges on the protein surface that interacts with the DNA molecule. The molecular electrostatic potentials for the DNA, simulated as a discontinuous cylinder, were calculated using the values for all the base pairs. Computing these parameters, we calculated the relative energy of interaction and the more stable rotational setting of DNA. The binding of four molecules of netropsin to this model showed that a new minimum of energy is obtained when the DNA turns toward the protein surface by about 180°, so a new energetically favoured structure appears where netropsin binding sites are located facing toward the histones surface. The effect of netropsin could be explained in terms of an induced change in the phasing of DNA on the core particle. The induced rotation is considered to optimize non-bonded contacts between the netropsin molecules and the DNA backbone.

INTRODUCTION

The nucleosome core particle is the fundamental structural subunit of eukaryotic chromatin [1,2]. The experimental evidence suggests that the nucleosome core is a flat disk where about 140 base pairs of DNA appear to form a superhelix wrapped around the histones core.

The major and minor grooves of the DNA, which is in a right-handed β-helix, can be located in the electronic density map [1], thus defining the DNA superhelix. The widths of the major and minor grooves seem to depend on the degree of bending, hence the minor groove appears to be less compressed than the major groove inside of the curved regions[1,3−5].A dynamic aspect of DNA wrapped on the core particle is the degree to which the polynucleotide is constrained from free rotation on the surface of the nucleosome. In fact, the DNA path is not smooth [1], and there are substantial contacts of DNA with the protein on one side of the helix [6]. Lysine amino groups that are closely associated with DNA have been identified by protein protection against chemical modification [6]. Moreover, other amino acids can interact with the polynucleotide.

In general, certain sequence-dependent modulations in structure appear to determine the rotational phasing of DNA about the core particle [3−5], thus the positioning of DNA about the histones octamer would depend largely on the anisotropic resistance of DNA to bending. That preference is rather weak and therefore could be modified by regulatory elements [2], so to influence the control of several cell processes.

Patterns of sensitivity to DNAse I cleavage have been analysed in attempting to understand the effect of several anti-tumour antibiotics on the structure of nucleosome core particles [7−10]. The effect of several antibiotics and related drugs, which includes bis-intercalators, as well as minor-groove binding ligands [7−10], but not mono-intercalators [8−10], can be satisfactorily explained in terms of induced change in the phasing (rotational setting of DNA on the core particle). The simplest interpretation would be that the antibiotic have induced the DNA to change its rotational orientation with respect to the surface of the protein [7,10].

It remains unclear why the bis-intercalator echinomycin [11] causes the DNA to change its phasing on the protein surface, whereas the mono-intercalators appear unable to do so [8]. Since each echinomycin molecule bound to DNA unwind the double helix by about 48° [11] we might consider that the induced rotation (about 180°) of DNA reflects the unwinding process (p.e. after binding of 4 to 5 antibiotic molecules). However, knowing that very similar changes in phasing are observed with the minor groove binding ligands netropsin, berenil and distamycin, which are not able to affect significantly the helical winding of DNA [7,9,12,13] a different interpretation has been put forward [7−10]. The most likely explanation appears to be that the antibiotics bind to certain favoured sequences that are accessible

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on the outer surface of the DNA which is bound around the histones octamer [1–3] and induce them to turn inwards to face the protein surface, thus favouring the situation in which the major proportion of the stabilization the DNA-antibiotic complex arises from non-bonded contacts between the antibiotic molecules and the polynucleotide backbones.

The tight wrapping of DNA on the particle strongly suggests that the anisotropic flexibility of the polynucleotide may be an important determinant for the positioning of the core particle [3], thus A+T runs are preferently placed where the minor groove faces approximately inward towards the histones octamer. Nonetheless, when any particular DNA sequence is wrapped around the protein it is unlikely that all the bending preferences in that sequence will be satisfied. Hence, we should query about the meaning that a particular rotational setting of DNA, and its stability, might have from a thermodynamics point of view. In other words, we expect that any rotational preference of the DNA molecule on the core particle ought to be the one favoured energetically. Similarly, any changes in the rotational setting of DNA as those induced by the antibiotic netropsin [8] should be a 'new' energetically-favoured state, thus the changes in the orientation of DNA could facilitate the DNA-antibiotic interactions without disruption of the nucleosome core particle, as it is inferred from DNase I cleavage experiments [7–10,13]

METHODOLOGY

The present study was carried out by considering the electrostatic contribution to the interaction energy between the molecules involved. Several studies performed by different authors support the significance of this approximation when dealing with large molecules [14–18].

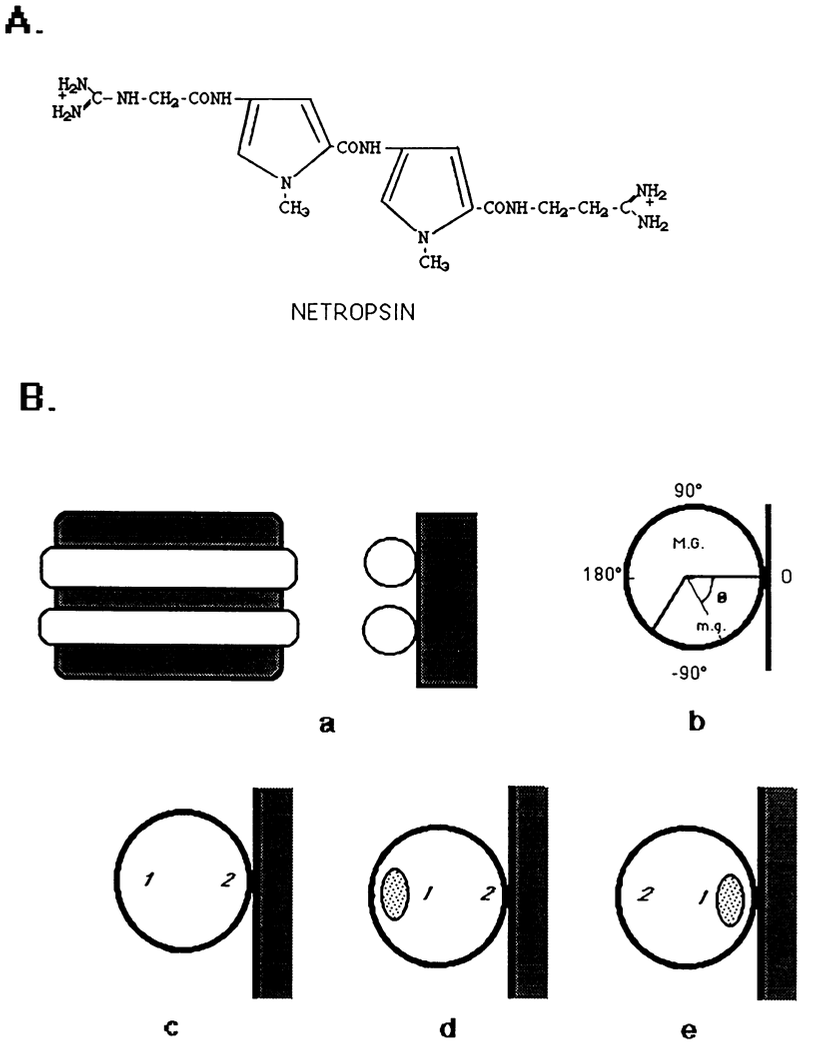

Figure 1. (A) Chemical formulae of netropsin. (B) Schematic views of a nucleosome core particle (a); definition of angle θ between the place of contact of DNA to the protein surface ($\theta=0°$) and middle of the minor-groove(m.g. in the figure, where M.G. states for major-groove). The values of θ became positive when going clockwise and negative when going anti-clockwise (b); A very schematic drawing of the energetically-preferred disposition of DNA on the core particle in the absence (c) and in the presence of netropsin (d and e). The numbers inside the figure are used to illustrate the change induced in the rotational orientation of DNA by antibiotic binding. A netropsin molecule is represented as a small patch on one site of DNA. The figure illustrates that the binding of netropsin to (c), as shown in (d), produces a new DNA setting displayed in (e).

Generation of a double-strand DNA and its map of theoretical Molecular Electrostatic Potential

The DNA molecule was modelled as a cylinder with a radius of 10.2Å. The cylinder was conformed by a set of disks spaced 3.3Å along the cylinder axis, thus to account for the sequence of the tyrT DNA fragment [3], see Fig.3. The disks were placed with a twist helical angle of 34.95° reproducing the periodicity of 10.3 base-pairs/turn observed in nucleosome core DNA [3–5]. The molecular electrostatic potencials for all the base-pairs were obtained from the isopotential curves described by Pullman and co-workers [15–16], and estimated at a distance of 10.2Å without shielding by counterions. This procedure renders a cylinder-like structure formed by disks representing all the base pairs of the tyrT DNA molecule separated by 3.4Å. The twist angle between the base pairs was fixed as 34.95°, thus to produce a periodicity of 10.3 base-pairs per turn.

Simulation of a nucleosome core particle and the DNA-protein interaction

The relatively low crystalographic resolution of the core particle [1] does not allow us to know the exact contacts between the amino-acids and any particular base pairs. However, the histones octamer can be modelled as a wall with a non-uniform distribution of charges, where the DNA cylinder is attached, see Fig.1. It is still possible to obtain a reasonable disposition of positive charges on the surface of the protein wall when we simulate them by a discontinuous arrangement of the net positives charges which are known to contact to the DNA helix [6,19]. We considered the experimental data on the relative position of lysines and arginines in the protein octamer [6], as well as, the DNA-protein contacts [19]. Therefore, the model computed the tentative assignments of the DNA-histones contacts in the nucleosome core particle provided by Bavykin et al. [19], using the net positive charges being uniformly spread over the regions where the DNA-protein contacts are located.

A program was written in FORTRAN and run on an AT-computer in order to calculate the relative energy of the system for different orientations of the DNA molecule once it is docked on the protein surface. The program evaluates the relative energy of the interaction between the protein charges and the molecular electrostatic potentials of DNA. A vacuum electrostatic constant was used in the present calculations. In fact, the presence of the solvent in our calculations would increase the dielectric constant, thus lowering the electrostatic interactions. This event would modify the numerical values obtained, but it does not affect the comparison of the relative energies involved in the process. Following our approach the minimum value will correspond to the most stable rotational setting of the polynucleotide after the protein surface. For the seek of convenience, the angles (0 to 180°) in respect to the protein wall were considered negative when going clockwise and positive when going anti-clockwise (see Fig.1B). Once the more stable docking of DNA is obtained, the computer program generates a list of helical twist angles for the whole list of base pairs of the DNA molecule. This angles give information about the position of the minor-groove of DNA, i.e. which grooves of the polynucleotide face out and which parts face in toward the protein surface.

Generation of a netropsin molecule: Molecular modelling analysis of its interaction with the nucleosome core particle

Netropsin molecules were generated from crystalographic parameters [12,20] using the Alchemy program [21]. The set of atomic coordinates were conveniently transformed to the local coordinates system of the DNA molecule. The electrostatic potential for the antibiotic molecule was evaluated from the atomic charges which are given in ref. 17. Two sets of calculations were performed to model the interactions the tyrT DNA and the nucleosome core histones. First, the DNA molecule was docked on the protein surface and the interaction energy evaluated for the different orientations of the DNA cylinder. Second, netropsin molecules were inserted near the cylinder surface covering tracts of at least 4 adenines or thymines, which are known to be the specific binding sites [12,13,18,20,22], see Fig. 1. Since netropsin binds through the minor-groove only the sequences where the minor groove lies facing outside the core particle can, in first instance, act as binding sites [8,10]. Once four to five netropsin molecules were fitted snugly into the DNA minor-groove [12,20]. A new computation of the relative energy of interaction was performed and the energetically preferred angle between the DNA and the protein surface calculated. This molecular modelling approach allows us to compare the angle between any DNA and the histones octamer in absence and presence of netropsin, thus we can estimate whether it fits with the experimental results and calculate any change in the tyrT DNA phasing on the core particle induced by netropsin binding.

RESULTS

A plot of the relative interaction energy between the tyrT DNA and the histones octamer is shown in Fig.2. The plot displays the values of the angle (θ) between the centre of the minor groove and the protein surface as described in Fig.1. The minimum energy (−297.2 Kcal/mol) corresponds to an orientation angle of −144° at the first base pair considered (namely number 13 of the tyrT DNA sequence shown in Fig.3), so its minor-groove is approximately facing outward the protein surface. The values of the angle θ for the rest of the polynucleotide is obtained by adding to this first value the helical twist angle of 34.95°, which corresponds to 10.3 b.p.(base pairs)/turn, that is the periodicity

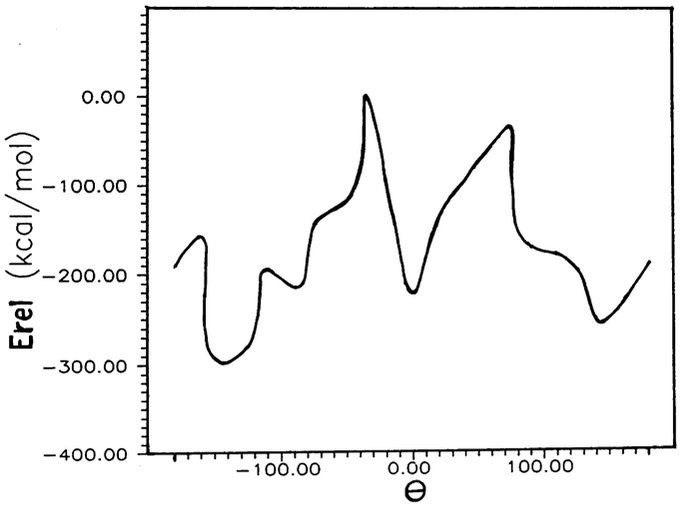

Figure 2. Plot of the relative energy of interaction *versus* the angle (θ) which meaning is shown in Fig. 1. The figure evidences that an angle of −144° corresponds to the more favoured orientation for the first base pair considered in the molecular modelling analysis (Erel= −297.2 kcal/mol).

of DNA in a core particle [3,4]. Hence, we can calculate the relative setting of each base-pairs (in fact, the centre of the minor groove) in respect to the protein surface, thus we deduce which regions of the DNA minor-groove face outward and which face toward the protein surface. By use of this method we can assign positions 14, 24, 35, 45, 55, 66, 76, 86, 96, 107, 117, 127 to the outer surface of the DNA helix. Fig 3 displays a plot representing ($-\cos\theta$) *versus* the base-pair number. This is a representation of the location of the minor-groove of DNA in respect to the protein wall. Positive values mean that it faces outward, while negative values that it faces toward the histones octamer. In Fig. 3 we also indicate the positions which would correspond to the sites more susceptible to DNAse I cleavage in reconstituted nucleosome core particles containing the tyrT DNA fragment [3].

Indeed, there is a clear correlation between the outside-facing orientation of the centre of the minor groove in the energetically preferred orientation of DNA and the DNAseI cleavage results [3–5]. The small differences might be atributed to irregularities in the path of the DNA as it winds around the protein[1]. An additional possibility is that the access of DNAse I to the minor-groove is somewhat hindered in the centre of the core particle, so other factors than the angle of the minor-groove can influence the exact rate of cleavage. In any case, it is evident that the energetically favoured orientation and the DNAse I cleavage pattern are strongly related. Therefore, it seems that our molecular modelling study renders the more energetically-favoured azimuthal orientation of the DNA cylinder, thus the molecular modelling procedure described here produces results which are equivalent to those reported by DNAse I cleavage on nucleosome core particles [3], that is the orientation of the DNA molecule favoured by the anisotopic bending of DNA. Although, in Fig. 3, we only represent the bases between positions 13 and 135 it is noteworthy that the characteristic modulation of the DNA cylinder will continue through regions on either end.

Once we have shown how the rotational setting of DNA after the core particle can be satisfactorily modelled, we simulated by molecular modelling procedures, as described above, the interaction of several netropsin molecules to a nucleosome core particle. In Fig.4 we show a plot representing the relative energy of interaction between the tyrT DNA and histones octamer in the presence of four netropsin molecules. As in Fig.2 the plot

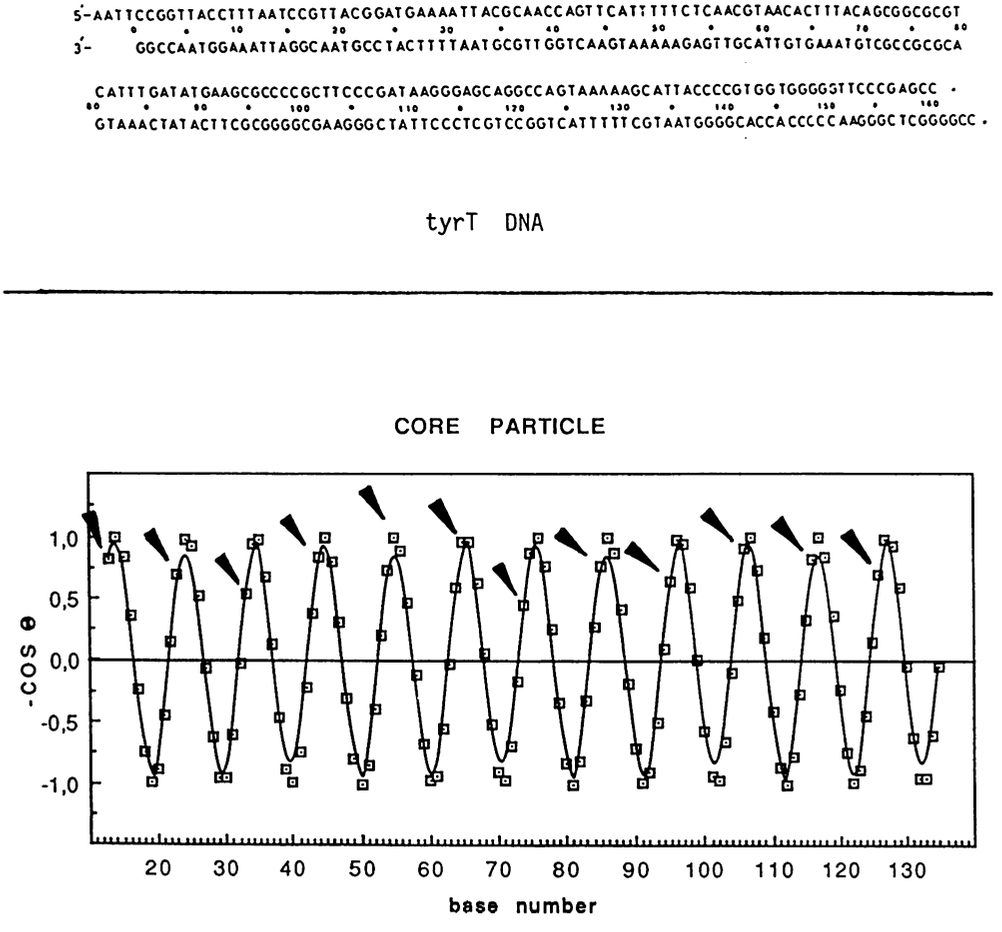

Figure 3. Nucleotide sequence of the tyrT DNA [3]. Plot representing the values of $-\cos\theta$ (the meaning of this angle is given in Fig 1) *versus* the base pair numbering of the tyrT DNA, whose sequence is displayed at the top of the figure. The plot shows the minor-grooves of DNA which are facing toward the nucleosome core surface as a negative value, while positive values represent the DNA minor-groove facing outward the protein surface. The arrows in the plot show the base pair more susceptible to DNAse I cleavage as deduced from the nuclease probing on nucleosome core particles [3,7–10]. A clear correspondence between the plot deduced from the molecular modelling study and the DNAse I susceptibility of the DNA on the core particle is observed.

displays the values of the angle θ between the centre of the minor-groove for the first base pair (namely number 13 in the sequence displayed in Fig.2) and the protein surface.

Netropsin molecules were carefully fitted in regions which are at least four base-pairs long. These regions where selected to be both preferred binding sites [12,13,18,20,22] and located facing outside the histones surface,[3,7,8] and Fig. 3.

In the presence of four netropsin molecules, fitted into the minor groove at the following A+T tracts: 47−50, 66−69, 82−85 and 126−129, we observed a new phasing of DNA induced by antibiotic binding. The new rotational positioning appears to be almost independent of the number of netropsin molecules bound to the polynucleotide model. The exact number

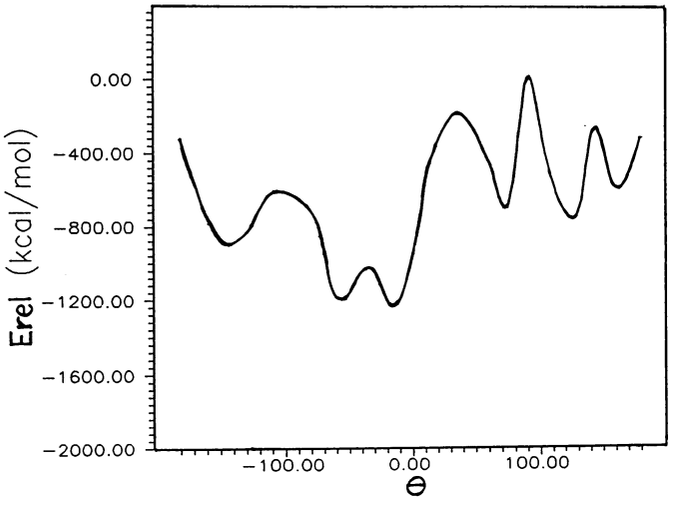

Figure 4. Plot of the relative energy of interaction (in kcal/mol) *versus* the angle (θ) between the protein surface and the centre of the minor-groove of DNA, in the presence of four molecules of netropsin bound to the polynucleotide. The figure evidences that an angle of −18° corresponds to the more favoured orientation in the presence of netropsin (Erel = −1235.3 kcal/mol). Other details as in Fig. 2.

and disposition of netropsin binding sites only changes slightly the apparence of plots like the one displayed in Fig. 5 which represents the binding of four netropsin molecules. In the presence of the antibiotic we can assign the minor-groove centre of the base-pairs 18, 28, 39, 49, 60, 70, 81, 91, 102, 112, 123 and 133 to the more outer surface of DNA. These regions which can be seen as maxima in the sinusoidal curve in Fig. 5 are approximately those observed facing inside, i.e. the minima, in Fig.3. If we compare both figures the simplest interpretation is to consider that netropsin has caused the DNA cylinder to change its rotational orientation with respect to the surface of the protein by about 180°.

Fig. 5 also displays the nucleotides which are known to be more susceptible to DNAse I cleavage in nucleosome cores bound to netropsin [8]. The conspicuous new bands in an electrophoresis gel of nucleosome core particles digested by DNAse I in the presence of netropsin [8−10] nicely agree with a higher exposition detected in the energetic calculations, which correspond to DNA minor-grooves facing outward the protein surface.

The new phasing of DNA on the histones octamer has an intertaction energy of −1214.1 kcal/mol, thus the change in the rotational orientation of the DNA on the surface of the protein is clearly favoured after netropsin binding.

The calculations for the model containing histones DNA and netropsin were performed taking either a period of 10.3 b.p./turn (the value calculated for DNA on a native core particle [3,4]), or 10.5 b.p./turn, which is the period calculated by Fourier analysis of netropsin-induced differences in susceptibility of nucleosome cores to DNAse I digestion [8]. We observed that in both cases the relative energy of interaction is similar. However, when we compared the preferred rotational setting of the DNA cylinder we observed that with a period of 10.5 b.p./turn we obtained a better fit between the DNA setting deduced from DNAse I cleavage [8], and the rotational orientation which is energetically favoured. We will analyse the meaning of this observation in the following discussion.

In any case, the molecular modelling approach presented here renders results that clearly hold the hypothesis previously put

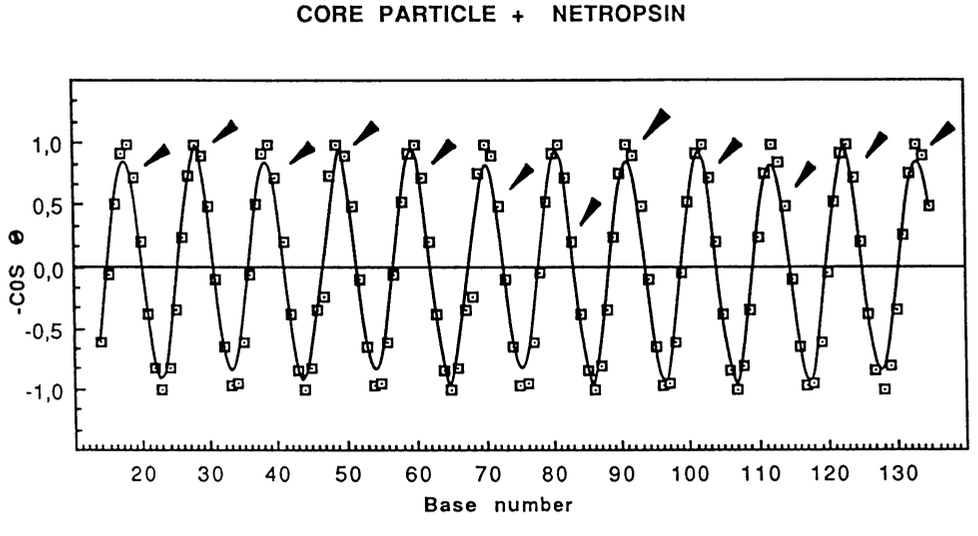

Figure 5. Plot representing the values of −cos(θ) *versus* the base pair numbering of tyrT DNA, whose sequence is shown at the top of Fig 3. The plot displays the results obtained when four netropsin molecules are bound to the nucleosome core. Other details as described in the legend to Fig. 3.

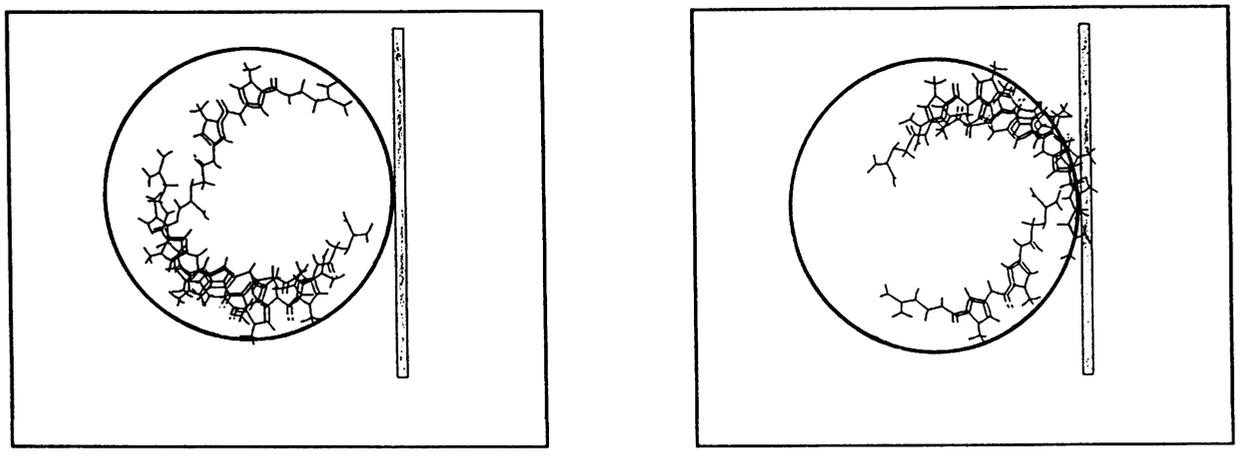

Figure 6. A view through the DNA helical axis of two computer generated drawings which show the spacial disposition of four netropsin molecules bound to a DNA molecule wrapped on the core particle. The left panel shows the location of the antibiotic molecules before the DNA has changed its rotational orientation, while the right panel shows them when the new rotational (energetically-favoured) setting is attained.

forward that the binding of certain minor-groove binding ligands [7–10] can change the rotational setting (phasing) of DNA to a new core structure where the ligand binding sites 'prefer' to be facing inward close to the protein surface (see Fig.6), thus producing a rotation of the DNA molecule by about 180°.

DISCUSSION

We have clearly shown that the rotational setting of a DNA fragment on the nucleosome core particle can be satisfactorily determined by molecular modelling when we compute the interaction energy by means of the molecular electrostatic potential for each base pair [15], and the distribution of protein charges in the regions of the histones which are tentatively supposed to contact the polynucleotide [6,19]. Moreover, after netropsin binding a new energetically-preferred orientation arises which nicely agrees with a change in the rotational setting of DNA wrapped on the core particle, so as to place the antibiotic binding sites on the inward-facing surface of the DNA superhelix (see Fig.6).

Despite of the fact that these results speak for themselves it might seem at first sight unlikely that the DNA molecules would lie in different orientation on the core particle after netropsin binding without generating a nucleosome core disruption. Let us see why this does not seem to be the case.

The possibility of inducing rotation of DNA on the surface of the core particle is supported by an analysis showing the presence of DNA motion in nucleosome core particles [23]. This theoretical analysis has convincely shown that the DNA after the nucleosome, though behaving as if rigidly clamped, may enjoy substantial rotational mobility, thus it should be relatively free to rotate, for example after netropsin binding.

Drew and McCall [5] have estimated the range of energies required to rotate various DNA molecules through an angle of 180° between 2.3 and 5.6 kcal/mol. Since these energies are above the thermal excitation value, they concluded that DNA positioning about the histones octamer is a stable one. Their model considers the steric hindrance for DNAse I to access the double strand and that the restoring force is like that of a spring supporting all possible distortions to give a equally stable protein-DNA complex. Although their energy values are not strictly comparable with the calculations presented in this paper (since molecular energy interactions are not straightforward comparable to bulk free energy), we are of the opinion that scaling both results conveniently, for the seek of comparison, the effect of adding several netropsin molecules provides a much more stable phasing when the polynucleotide is rotated by about 180°, compared to the situation without bound antibiotic molecules. The antibiotic binding would supply the energy required to circumvent the energy barrier associated with rotation whatever the way or relative units we use to calculate it. In any case, our model has the advantage of considering the protein charges effect and protein-DNA interactions on any rotational setting.

In the core particle, DNA is bent into a supercoil of radius 43Å [1,2] and its positioning depends largely on the anisotropic resistance to bending stress. Since we have shown that this positioning after the histones octamer reflects a energetically-favoured orientation, we analysed the effect of DNA bending on the energies of interaction. Estimates have been made of the energy required to bend DNA into a continuous supercoil after the histones octamer using the relationship between the persintence length of DNA and its average bending constant [24]. It seems that about 20–28 kcal/mol are required to bend the DNA after the nucleosome core particle. It can be estimated that the DNA bending contribution to the total energy of the system will be hardly different in both states, being only involved in the kinetics of the rotational change.

Our results have been obtained considering that the contacts between the DNA and the protein are preserved during the change in the phasing of DNA after the core particle (though they do not need to be exactly the same in both DNA settings). It seems from both the experimental results on DNAse I cleavage of core particles in the presence of netropsin [8] and the molecular modelling study presented in this paper that an increase in the helical repeat (say from 10.3 to 10.5 b.p./turn) facilitates the new DNA setting after the antibiotic binding.

The change in helical repeat could reflect a certain degree of deformation in the core particle, which may be needed in order to obtain a new core particle where the DNA has rotated by about 180°. This change may be to a nucleosome unfolded structure,

as it has been observed when ethidium binds to core particles [25], thus to preserve DNA-protein interactions during the kinetics of the process. Alternatively, the increase in helical repeat could be related with the linking number of a surface wrapped DNA [26], so the change in the winding number (the number of base pairs divided by the average helical repeat) changes to keep the surface linking number invariable. We have found, [8] and this article, that in the presence of netropsin there is an increase of about 0.2 b.p./turn (10.5 minus 10.3) in the helical repeat of the tyrT DNA wrapped on the protein surface. In fact, a change in the helical repeat of 0.1 b.p./turn would account for the experimental results if the DNA-protein contacts are not preserved [27]. The value of 0.2 b.p./turn observed both in DNAse I cleavage experiments [8,10] and in the molecular modelling study might well represent that an important proportion of contacts are not significantly altered during the netropsin-induced change in DNA phasing on the nucleosome core particle.

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