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Hypothesis

A model for the ability of drugs to induce enhanced DNase I cleavage

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A common property of sequence-selective DNA-binding drugs lies in their ability to induce an enhanced DNase I cleavage in regions surrounding their binding sites. A hypothetical model to explain the enhancements induced by drug binding to the minor-groove of DNA is presented. It involves the participation of three different single models: a mass action effect produced by the enzyme redistribution after drug binding; changes in the minor groove width size; and interactions between the enzyme and the drug, so increasing the cleavage in places located close to the binding site. The model is tested by using statistical data analysis.The hypothetical model might explain the experimental results better than any of the single models alone, but these models also appear to render significant results.

DNA-drug binding; Netropsin; Footprinting; DNase I cleavage enhancement

1. INTRODUCTION

Footprinting is a technique widely used in the analysis of sequence specificity of drugs which bind to DNA. The experimental approach is based on the ability of a DNA-bound ligand to protect its binding site(s) from enzymatic (or chemical) cleavage. Footprinting experiments have been employed to locate the binding sites for several drugs on different DNA fragments ([1–9] and references therein). Moreover it is also evident that the rate of cleavage at certain bonds is strongly enhanced relative to that in the control [1–9].

Although DNase I is not the only cleaving agent available, this nuclease appears to have several properties that recommend it as an accurate footprinting tool [3,6,10–12]. Its mechanism of binding to and cutting DNA is well established [10,11,13].

Because after drug-binding several DNA regions are protected from DNase I cleavage, it could be suggested that the enhancements in DNA cleavage are due to the restricted linear diffusion of the enzyme on DNA, since the binding sites are blocked by the drug molecule. This situation (see fig.1) is considered a mass action effect that should provide us with a footprint where the non-protected DNA bonds would become equally enhanced. However, the analysis of published footprinting patterns [1–9] shows that this situation is not apparently observed, since in several cases, a clear enhancement appears in some of the DNA bonds, while others remain as in the original control lanes [1–9].

Two other theories can be put forward to explain the presence of DNA cleavage enhancements: (i) we can suppose that the enzyme exhibits some affinity toward the drug molecule which is already bound to DNA, thus it would cut more effectively at sequences close to the binding sites [14,15]. (ii) A different model would suggest that the enhanced digestion may occur due to a conformational change in DNA brought about by the sequence-selective binding of drugs to the polynucleotide [3,4,6].

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A common feature of sequence-selective binding drugs that intercalate between DNA base-pairs is the ability to unwind the DNA, so a more or less pronounced degree of unwinding [16] may produce the effect of opening up the grooves at neighbouring sequences [3,17]. It is noteworthy that some sequences appear to be more affected than others.

Diethylpyrocarbonate (DEPC)-mediated DNA strand cleavage in the presence of different *mono*- and *bis*-intercalators confirms the ability of some compounds to alter the DNA structure in a sequence-dependent manner [9,18–22]. The altered structures can be observed either proximal or distal to the drug-binding sites [18,19,21], a situation that is consistent with drug-induced structural changes in DNA [22].

We should query if these results agree with an enzyme-distribution mechanism. Although both situations could exist together, it is to be noted that mass action effects alone might not explain the general levels of enhancements observed in footprinting experiments using intercalating drugs [3–5]. The second model requires the enzyme to exhibit some affinity for the drug molecule, being attracted to the bound ligand, so that it would cut more rapidly at sequences close to the binding sites. In general, this situation seems quite unlikely because the conformation of some drugs is such as to present few donor-acceptor groups for binding to the enzyme [3,16].

2. THE ORIGIN OF THE ABILITY OF MINORGROOVE BINDING DRUGS TO INDUCE ENHANCED RATES OF DNase I DIGESTION

While for ligands that intercalate between DNA base-pairs the enhanced cleavage patterns appear to be mostly due to a conformational change in DNA, the situation with the minor-groove binding drugs remains less clear [14,15].

In this article, I present a model to explain the DNase I cleavage enhancements after binding of drugs to the minor-groove of DNA. The hypothetical model, that will be tested using linear regression methods [23], considers that the enhancements observed in the footprinting experiments [1,6–8,14] are produced by the combined action of the three models described above.

Although changes in the minor-groove width could explain the increment in cutting rates at several bonds both proximal and distal to the drug-binding sites [4,6,8], this simple model has a serious handicap since the minor groove binding ligands only produce relatively small changes in the structure of DNA [24–26]. Enhancements have been suggested to arise from a drug-induced redistribution of the enzyme [14,15], with higher enhancement values for sites adjacent to binding sites, but this proposal does not explain the enhancements which are not in the vicinity of a binding site [6,8]. Data about whether the drugs that bound to DNA also bind to DNase I are scarce [3,12,14]. As a matter of fact, the drug-enzyme interaction is supposed to be different in the presence from that in the absence of DNA, since some of the potential drug-enzyme binding sites are involved in DNA-drug interactions. In spite of this, netropsin molecules bound to DNA could still form hydrogen bonds to the enzyme through parts of the molecule which do not appear to be involved in hydrogen bonding to DNA [25].

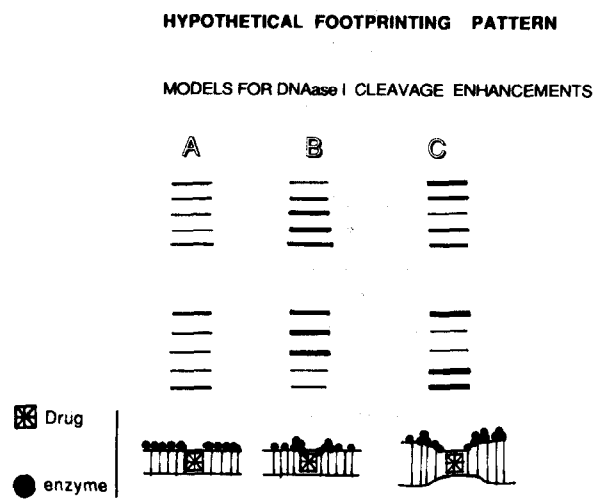

Fig.1. Illustration of hypothetical footprinting patterns produced by DNase I cleavage of drug-DNA complexes in three different situations. Sketchily, the figure displays both the cleavage model and the patterns that it should generate. (Model A) Mass action model. After drug binding the enzyme cleavage is enhanced at places which are not binding sites. (Model B) The nuclease digestion is enhanced at places close to the drug-binding sites. There is a drug-enzyme interaction. (Model C) After drug binding the DNA molecule undergoes a structural change that makes it more susceptible to the enzyme in several places both proximal and distal to the drug-binding sites.

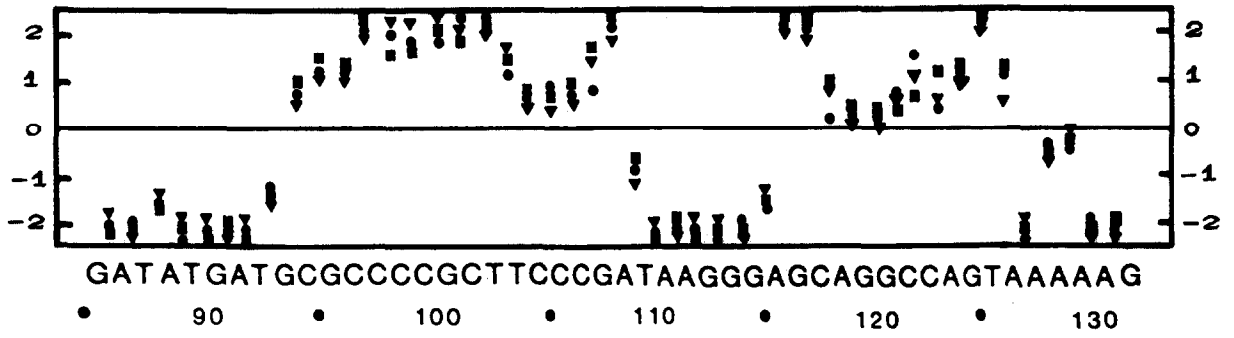

Fig.2. A map of differential cleavage values (logarithmic scale) generated to simulate the hypothetical models A (▼); B (■); and C (●), described in fig.1. The differential cleavage values were calculated with respect to the tyrT DNA fragments (bonds 86 to 131), thus correcting them for the characteristic DNase I cutting behaviour [10,11].

3. TESTING THE MODEL: A SIMULATION APPROACH

Footprinting results obtained on the binding of netropsin to the tyrT DNA fragment [6] were compared with those theoretically generated using the following approach: three different digestion patterns, related with panels A, B and C in fig.1, were created. A hypothetical DNase I digestion was simulated on a sequence-averaged DNA fragment. For purposes of comparison with the experimental results [6], the fragment was chosen to contain two netropsin-binding sites, which were similar in size and location to those found experimentally in the tyrT DNA between nucleotides 86 and 132 (see fig.3). A randomly selected integer number was used to represent theoretically a radioactively labelled DNA, i.e. the total quantity of radioactivity of the DNA fragment. To simulate the DNase I cleavage the integer was divided between the 46 bonds (i.e. the number of bonds between nucleotides 86 to 132), taking care to simulate the drug-binding sites with a small integer. The numerical quantities that were not used to represent the cleavage at protected sites were then reallocated to the drug-free bonds in order to simulate the enhancements. This was performed either randomly (model A in fig.1); accumulating the amounts in bonds close to the binding sites (model B); or in bonds located at intermediate places between drug-binding regions (model C), thus simulating any one of the three hypotheses described above (see fig.1). Data from the computer-simulated experiment are presented in the form of $\ln(f_{drug})-\ln(f_{control})$ (fig.2) which represents the differential cleavage at each bond relative to that in the control (DNase I cutting in the absence of drug) [3–8]. The results are shown

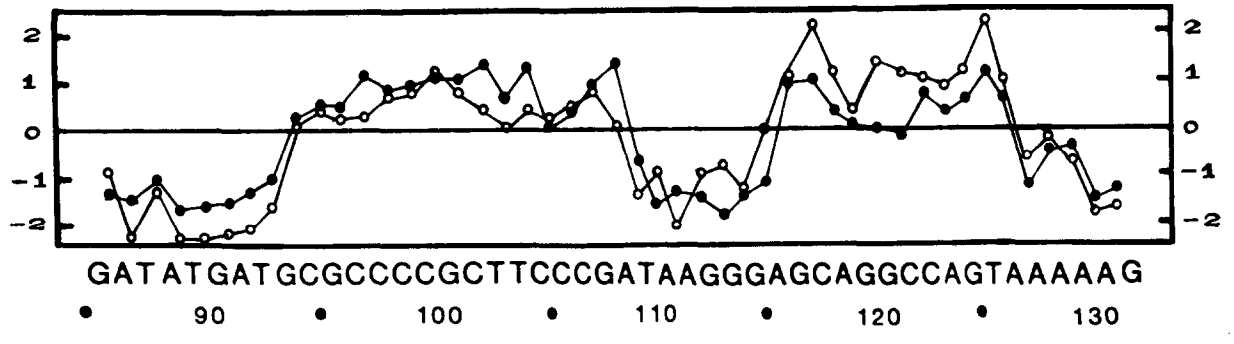

Fig.3. Plots of differential cleavage representing the effects of netropsin (○), and the hypothetical values generated by linear regression methods from models A, B and C (●) described in figs 1 and 2. Experimental data taken from [6].

on a logarithmic scale for convenience of plotting. Positive values indicate enhancements, negative values blockage.

A statistical data analysis using linear regression methods [23] was undertaken to evaluate the adequacy of each model, taking the theoretically predicted radioactivity values as the independent variable and the experimental results as dependent variable [6]. The study was performed by using a GW-Basic computer program developed by the author from mathematical algorithms reported in [27]. All calculations were carried out on an Amstrad PC-1512SD computer. Fig.3 shows a comparison between an experimental cleavage plot [6] and a plot calculated for the hypothetical model.

4. DISCUSSION

The analysis of all the hypothetical model shows (table 1 and fig.3) that about 70% of the DNase I cleavage enhancements could be produced by a combined action of the three simple models in figs 1 and 2 ($r^2(A+B+C) = 0.7096$), but also for any of the single models alone (A, B or C in fig.1 and table 1). It is to be noted that the variance explained by the different models is similar in all the cases displayed in table 1. The hypothesis of a triple (combined) effect (mass action, DNA conformational change, and enzyme-drug interaction) cannot explain all the observed enhancements. The same could be said about any of the simple models. Nevertheless, certain improvement of the correlation coefficient appears when a multivariate effect is considered (see table 1).

Let us consider how the three effects can act together, since we can barely distinguish between them. The mass action effect is independent of the DNA sequence and is due to the linear redistribution of the enzyme after drug binding. It reflects an equal distribution of DNase I molecules which are unable to cleave the drug-protected regions. It is inferred that a mass action effect will always be present. Although changes in DNA structure do not appear to be as spectacular as with intercalators [3,16,19] they still exist as shown by the overwinding effect of netropsin [24] and the ability of the drugs to change the minor-groove size [25,26]. DNase I cleavage of double helix DNA involves the binding of the enzyme to an exposed minor groove [13]. It is possible, therefore, that the nuclease may interact with netropsin molecules which are located in the AT-rich minor-groove [6,14,25,26].

Table 1 shows that the multiple action model is statistically significant. Nevertheless, it cannot explain all the experimental results [6]. Probably, there is an effect of flanking sequences. The hypothetical models used in the simulated computation were built assuming an averaged DNA sequence, except for the netropsin-binding sites (A+T rich regions at least four base-pairs long [1,6]), but in the tyrT DNA fragment – or indeed any natural DNA – the sequences flanking the binding sites are more or less different, thus affecting DNase I cleavage either in the presence of ligands [13,17] or their absence [11]. For example, in fig.3 the differential cleavage plots are clearly different in the region between bonds 97 and 107. This is a pyrimidine-rich region (10 pyrimidines among 11 bases, against 19 among 46 in the entire

Table 1

Comparison between data obtained in a netropsin DNA-interaction by simulation processes and experimental results

	Simple correlation				Multiple correlation		
Model	r	r^2	t	Model	r	r^2	F
A	0.8377	0.7017	10.172	A+B	0.8423	0.7095	52.514
B	0.8422	0.7093	10.361	A+C	0.8391	0.7040	51.139
C	0.8356	0.6982	10.089	B+C	0.8424	0.7096	52.524
				A+B+C	0.8424	0.7096	34.214
	($n=46$; $p<10^{-4}$)				($n=46$; $p<10^{-4}$)		

'Model' refers to different theoretical models for DNase I cleavage enhancements after drug binding, as described in figs 1 and 2. r is the Pearson's correlation coefficient and r^2 the variance explained by the model. F is the Snedecor's F-value and t the Students-t value

fragment). A peculiar conformation of this DNA region could explain its different behaviour, either by changes in DNA conformation after drug binding and/or by affecting the interaction between the bound drug and the enzyme. In fact, poly(dA)·poly(dT) or poly(dG)·poly(dC), which contain a pyrimidine polynucleotide strand, are known to be structurally abnormal and essentially straight [28], so they are, for example, able to resist reconstitution into nucleosomes [29–30]. The Levene and Crothers model for DNA bending [31] proposes that poly(A) runs adopt a non-B conformation and there is a marked change in direction of the helix axis at the junction between B and non-B DNA regions. Furthermore, DNase I covers at least four base pairs at the 5′- and six base pairs at the 3′-side [13], so the bulk of DNase I can produce a different pattern on either side of the binding region which cannot be easily introduced in a simulated model.

The footprinting technique allows evaluation of drug-binding constants [12,14,15]. The presence of DNase I cleavage enhancements away from the binding site needs to be considered in determining binding constants from footprinting data [14,15]. The correction of this effect during the quantitative treatment of data has assumed [15] that the enhancement pattern arises from a redistribution of DNase I molecules. The hypothetical model presented in this article shows that although this effect is significant it would be desirable to take into account that DNA structural changes are also involved in the enhancement patterns.

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