Volume 250, number 2, 323-327 FEB 07283 July 1989

Sequence selective binding of ditrisarubicin B to DNA: comparison with daunomycin

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Received 21 April 1989

DNase I footprinting has been used to examine the sequence selective binding of ditrisarubicin B, a novel anthracycline antibiotic, to DNA. At 37°C no footprinting pattern is observed, the drug protects all sites from enzymic cleavage with equal efficiency. At 4°C a footprinting pattern is induced with low drug concentrations which is different from that produced by daunomycin. The best binding sites contain the dinucleotide step GpT (ApC) and are located in regions of alternating purines and pyrimidines.

Daunomycin; Ditrisarubicin; Anthracycline; Footprinting; DNase I

1. INTRODUCTION

Ditrisarubicin B is a new member of the anthracycline group of antitumour antibiotics, possessing trisaccharides attached to both C7 and C10 of the anthracycline ring [1] (fig.1). It inhibits RNA synthesis at low concentrations [2] and binds to calf thymus DNA with an affinity ten times greater than the simpler anthracycline daunomycin [1].

Footprinting studies with daunomycin, performed at 4°C to stabilise the drug-DNA complex, have suggested that it binds to adjacent GC residues which are flanked by an AT base pair [3]. In the crystal structure of daunomycin complexed to d(CGTACG), drug molecules are intercalated into the CpG steps with the daunosamine ring positioned in the DNA minor groove [4]. The structure is stabilised by several hydrogen bonds. Theoretical calculations [5] suggest that the C9 hydroxyl group is involved in favourable interactions with the guanine above, and that the requirement for an AT base pair in the third position arises from repulsive

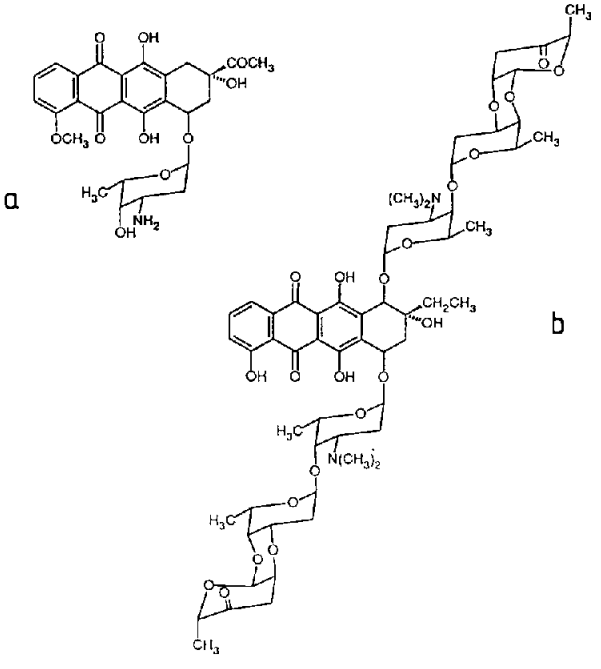

Fig.1. Structures of daunomycin (a) and ditrisarubicin B (b).

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

forces which would occur between the daunosamine and the 2-amino group of guanine.

In order to clarify the importance of these residues for the sequence selective binding we have performed comparative footprinting studies on ditrisarubicin B, which lacks the C13 carbonyl yet possesses an additional charged group on the first sugar attached to C10. The results show that ditrisarubicin B has different sequence recognition characteristics to daunomycin.

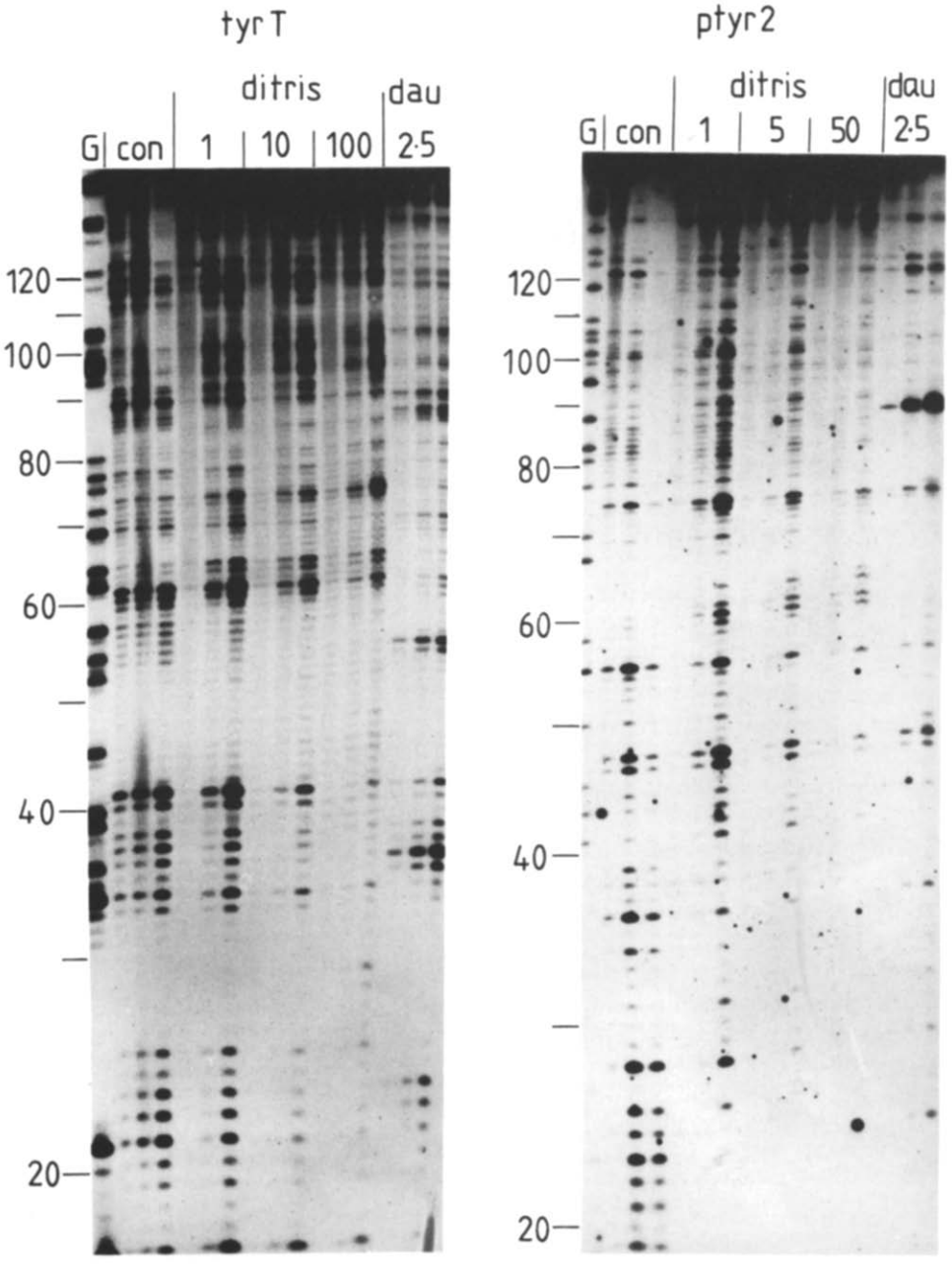

Fig.2. DNase I footprinting of the tyrT and pTyr2 fragments in the absence (con) and presence of ditrisarubicin B (ditris) and daunomycin (dau). The concentration of each ligand in micromolar is given above the gel lane. Each set of three lanes corresponds to digestion by the enzyme for 1, 5 and 30 min. The tracks labelled 'G' are dimethylsulphate-piperidine markers specific for guanine.

2. MATERIALS AND METHODS

2.1. *Antibiotics and enzymes*

Ditrisarubicin B was prepared as previously described [1]. Daunomycin hydrochloride was purchased from Sigma. Because of the unknown aqueous solubility of ditrisarubicin both compounds were dissolved in dimethyl sulphoxide (DMSO) and diluted to working concentrations in 10 mM Tris-HCl, pH 8.0, containing 10 mM NaCl immediately before use. The final concentration (always less than 10%) does not in-

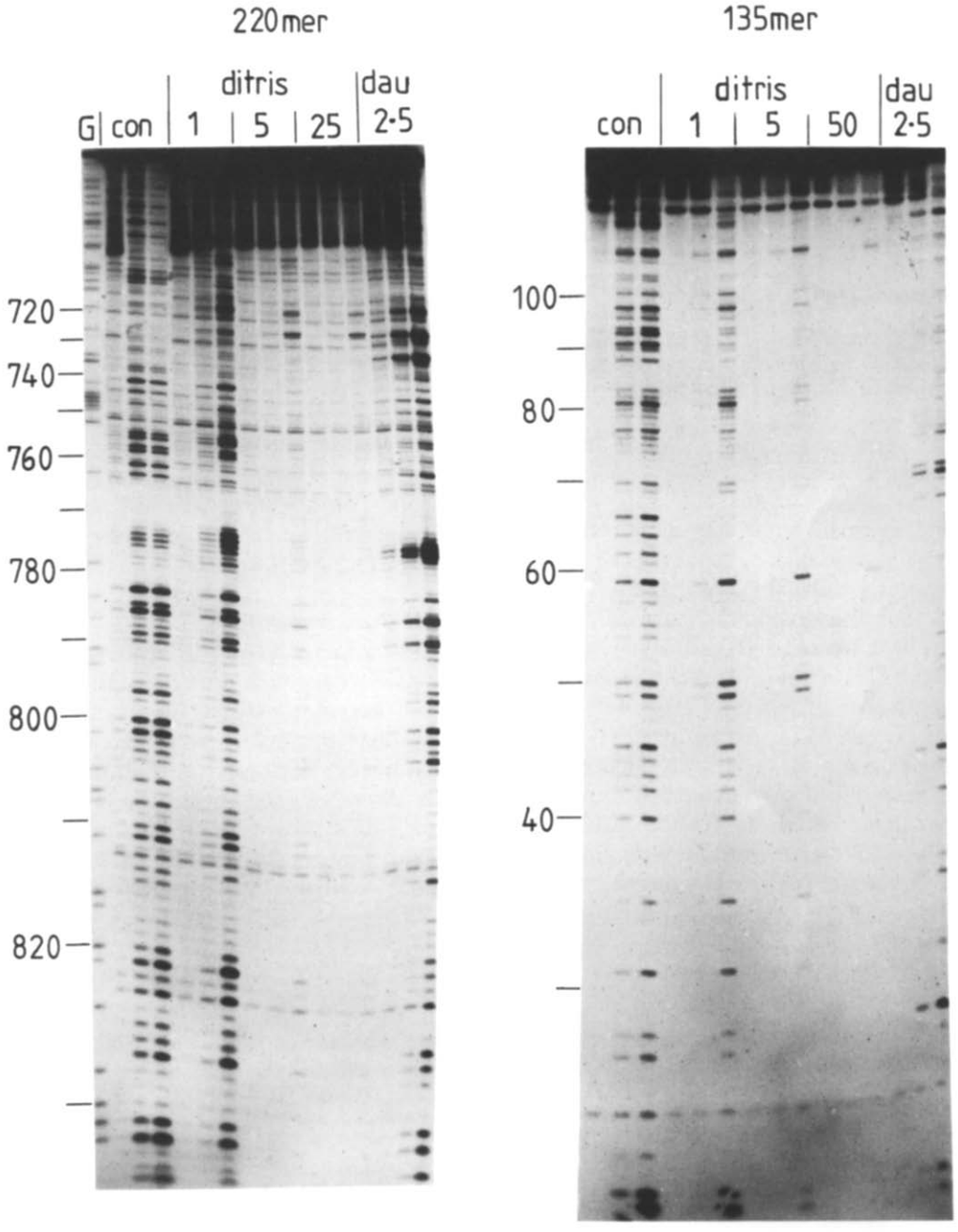

Fig.3. DNase I footprinting of the 135 and 220 base pair DNA fragments in the absence (con) and presence of ditrisarubicin B (ditris) and daunomycin (dau). The concentration of each ligand is given above the gel lanes. Each set of three lanes corresponds to digestion by the enzyme for 1, 5 and 30 min. The track labelled 'G' corresponds to a dimethylsulphate-piperidine marker specific for guanine.

terfere with the footprinting results and where a direct comparison with previous results for daunomycin is possible the footprinting patterns are identical. DNase I was purchased from Sigma, stored as a 7200 U/ml solution at −20°C and diluted to appropriate concentrations immediately before use.

2.2. *DNA fragments*

The four DNA fragments were isolated and labelled as previously described [6,7]. The 220-mer and 135-mer were prepared from plasmid pXbs1 [8] by cutting with *Hin*dIII, labelling at the 3'-end with [α^{32}P] dATP, cutting with *Sau*3A1 and separating the radiolabelled fragments on a 6% polyacrylamide gel. Both the 160 base pair *TyrT* DNA fragment and the 166 base pair ptyr2 DNA fragment were labelled at the 3'-ends of their *Eco*RI sites. The fragments were numbered as previously described.

2.3. *Footprinting*

Low temperature DNase I footprinting was performed as previously described [9]. The products of digestion were fractionated on 8% denaturing polyacrylamide gels. Bands in the digests were assigned by using Maxam-Gilbert G-specific sequencing lanes, or by comparison with previous DNase I patterns.

3. RESULTS

Footprinting experiments with ditrisarubicin B at 37°C failed to reveal any changes in the enzyme cleavage pattern, all bands showed a similar decrease in intensity. In contrast clear differences between the drug-treated and control lanes are apparent at 4°C. This effect of temperature probably reflects the kinetics of the drug-DNA interaction and has been observed for daunomycin [3] as well as other small ligands [9]. It is readily apparent that the two drugs produce very different footprinting patterns. At high concentrations both drugs protect all DNA bands form digestion, but at low concentrations a specific pattern is induced in which certain bands are protected while others show relative enhancements. The results are best explained by considering the two drugs separately.

3.1. *Daunomycin*

DNase I footprinting patterns in the presence and absence of 2.5 μM daunomycin are presented in figs 2 and 3 at 4°C. It is readily apparent that the drug has radically altered the enzyme cleavage pattern and a summary of the major sites of protection on the four DNA fragments is presented in table 1. It should be noted that at this low temperature there are several gaps in the control enzyme cleavage pattern, especially at homopolymeric runs of A and T, so that if the drug binds to any of these regions then it will not be detected; the sites listed in table 1 may therefore not include all the binding regions. DNase I is known to cut DNA in the minor groove and so produces cleavage patterns which are staggered by about 3 bonds towards the 3'-direction. Drug-binding sites should therefore be located at the 5'-ends of each of the protected regions. No simple consensus recognition sequence is apparent from these data, though many of these sites contain adjacent GC base pairs.

3.2. *Ditrisarubicin B*

Figs 2 and 3 reveal that at the highest concentrations ditrisarubicin protects nearly all DNA bonds from enzyme cleavage, suggesting that the ligand does not possess an absolute sequence binding preference. At lower concentrations, however, a few discrete bands are missing in each case. These are listed in table 1. With 1 μM drug specific bands are missing around position 760 on the 220-mer, 65 and 55 on the 135-mer and 20 on the ptyr2 DNA

Table 1

	Daunomycin	Ditrisarubicin
tyrT DNA	24–27 (TCATC)	19–25 (ATCCGTAA)
	31–34 (GTAAT)	35–40 (GGTTGCG)
	56–62 (GTTACGTT)	52–58 (CGTTGAGA)
pTyr2 DNA	19–23 (TGTAGG)	19–24 (GTGTAGG)
	38–44 (CGCGCATC)	
	77–86 (ATAGTGCACGA)	
220-mer	806–811 (AGGCCGC)	759–763 (CCATGC)
	777–782 (CCGAACA)	
	757–760 (CTCCA)	
135-mer	47–50 (AGCCT)	53–58 (GAGGGGA)
	55–65 (TGTAAGCCAGGG)	61–65 (TGTAAG)

fragment. In each of these instances the rest of the cleavage pattern is unaffected. These blockages are in different positions to those produced by daunomycin. As the drug concentration is raised a greater number of bands become protected. In contrast to daunomycin no drug-induced enhancements are apparent.

4. DISCUSSION

The results presented show that both daunomycin and ditrisarubicin B bind to DNA in a sequence selective fashion, and that the two drugs display different sequence preferences. The data for daunomycin are similar to those previously reported for the tyrT DNA fragment and are consistent with drug binding to two contiguous GC base pairs flanked by an AT pair. The best binding sites for ditrisarubicin often contain the dinucleotide step GpT (ApC) and are frequently located in regions of alternating purines and pyrimidines. In this regard it appears that the binding characteristics of ditrisarubicin B are more closely related to nogalamycin than daunomycin [6,7].

The inability of ditrisarubicin B to induce enhancements in the DNase I cleavage pattern is unusual. Most intercalating ligands increase the rate of DNase I cleavage in runs of homopolymeric A and T although this does not appear to be the case for daunomycin. These enhancements are understood as arising from regions of DNA to which the drug is not bound and which are structurally modified by ligand binding to adjacent sites. Two possibilities could account for the absence of enhancements with ditrisarubicin; either the drug does not perturb the DNA structure significantly, or more likely it only possesses a limited selectivity and binds reasonably well to those regions which would otherwise be enhanced.

The different sequence recognition properties of ditrisarubicin B were unexpected. It was anticipated that it should bind to similar regions of DNA having a larger binding site size on account of the extra sugar residues. The only site where this may be observed is around position 20 on ptyr2 DNA for which daunomycin protects as far as position 23 while the ditrisarubicin footprint extends to position 24. Generally the binding regions of the two ligands do not overlap. These binding differences may be due to the presence or absence of the C13 carbonyl group, the additional bulk provided by the trisaccharides, the second charged sugar residue or favourable contacts between the extra sugar residues and groups on the DNA. Since the binding sites for ditrisarubicin are similar to those observed for nogalamycin and related antibiotics [7] which also possess bulky sugar substituents it seems likely that the altered sequence selectivity arises from the nature of the sugar side groups, rather than hydrogen bonding substituents on the chromophore.

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