Z-DNA and other non-B-DNA structures are reversed to B-DNA by interaction with netropsin

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The interaction between the B-form specific ligands netropsin (Nt) and distamycin-3 (Dst-3) and DNA duplexes has been studied under conditions of salt concentration and low water activity that modify the polymer conformation into a non-B DNA form, putatively a Z-like form. Three polymers with strict alternating purine–pyrimidine sequences and GC content from 100–0% have been tested: poly(dG–dC)·poly(dG–dC), poly(dA–dC)·poly(dG–dT) and poly(dA–dT)·poly(dA–dT). The titrations by Nt and Dst-3 were followed by circular dichroism. Although specific binding of Nt to the Z-form of poly(dG–dC)·poly(dG–dC) does not occur, Nt reverses this Z structure to the B-type conformation; Dst-3 is, however, totally inefficient. The presumed non-B or Z-like structure of poly(dA–dC)·poly(dG–dT) is reversed to the B-form upon interaction with Nt; Dst-3 also induces this reversal but at higher ligand ratios. The modified B-structure of poly(dA–dT)·poly(dA–dT) in low water activity is efficiently reversed to the B-form by interaction with both Nt and Dst-3.

Z-DNA–B-DNA reversal *Netropsin* *Distamycin-3* *Antibiotic, small groove-binding* *Purine–pyrimidine alternation* *Polydeoxynucleotide–antibiotic pair* *DNA conformation*

1. INTRODUCTION

The small groove-binding antibiotics Nt and Dst-3 (fig.1) selectively interact with A·T base pairs in the right-handed B conformation of DNA [1–3]. These drugs show no significant binding affinity towards double-stranded RNA [1,4–6], A-type DNA [1,7] or most RNA–DNA hybrids [8]. Both drugs can also induce reversal from the A to B form of DNA [9–11]. We have shown that certain synthetic RNA–DNA and 2′-fluoro-2′-deoxy·DNA hybrids may also undergo the A to B-typetransition upon interaction with Nt and Dst-3 [12,13]. The three polymers poly(dG–dC)·poly(dG–dC), poly(dA–dC)·poly(dG–dT) and poly(dA–dT)·poly(dA–dT) possess a purine-pyrimidine sequence which is a requisite for the possibility of the B to Z transition. Under various ionic and solvent conditions, all of these polymers can be turned to different degree, to non-B DNA forms. Poly(dG–dC)·poly(dG–dC) adopts the left-handed Z-form under high ionic strength condi-

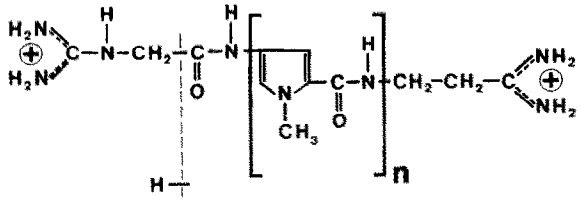

Fig.1. Chemical structure of netropsin (Nt; $n = 2$) and distamycin-3 (Dst-3; $n = 3$) lacking the left guanidinium group.

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Abbreviations: CD, circular dichroism; Nt, netropsin; Dst-3, distamycin-3

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tions [14–21] or by interaction with spermine [15]. Poly(dA–dC)·poly(dG–dT) may also form a left-handed structure in fibres [15]. In solution, this latter polymer clearly differs from the B-form [22,23] although the existence of a Z-type structure has not yet been proven. Poly(dA–dT)·poly(dA–dT) exhibits a conformation different from the normal B-form in high CsF concentrations [24]; however, a Z-type conformation has been ruled out for this so-called X-form [25].

The Z-form of poly(dG–dC)·poly(dG–dC), as well as the non-B DNA forms of poly(dA–dC)·poly(dG–dT) and poly(dA–dT)·poly(dA–dT) in reduced water activity conditions are all characterised by long wavelength negative CD bands. The Z to B transition can be therefore conveniently monitored by CD. Here, we report the interaction of Nt and Dst-3 with the 3 polymers poly(dG–dC)·poly(dG–dC), poly(dA–dC)·poly(dG–dT) and poly(dA–dT)·poly(dA–dT) under solvent and ionic conditions favouring the non-B DNA conformations; i.e., the Z-form for poly(dG–dC)·poly(dG–dC) and the modified non-B-forms for the two other polymers.

2. MATERIALS AND METHODS

Nt hydrochloride was a crystalline product [4] kindly donated by H. Thrum (Jena). Dst-3 was obtained from Boehringer, Mannheim. Extinction coefficients (in $M^{-1}.cm^{-1}$) [26]: Nt, ϵ_{296} = 21500; Dst-3, ϵ_{303} = 33000. Poly(dG–dC)·poly(dG–dC), poly(dA–dC)·poly(dG–dT) and poly(dA–dT)·poly(dA–dT) were purchased from Boehringer (Mannheim); the extinction coefficients [27] were ϵ_{254} = 8400, ϵ_{260} = 6500 and ϵ_{258} = 6800 $M^{-1}.cm^{-1}$. The binding ratio, expressed as ligand/phosphate (r'), is used throughout. CD spectra were recorded on a Jobin-Yvon Dichrographe III using 1 cm cuvettes.

3. RESULTS

3.1. *Poly(dG–dC)·poly(dG–dC)*

This polymer adopts the Z-form in 60% ethanol plus 1.5 mM CsCl [22]. The interaction of Nt and Dst-3 in these conditions can be followed by the change in the CD spectrum and is shown in fig.2. Interaction of Nt appears to be cooperative; at r' = 0.8, when the titration reaches a plateau, the

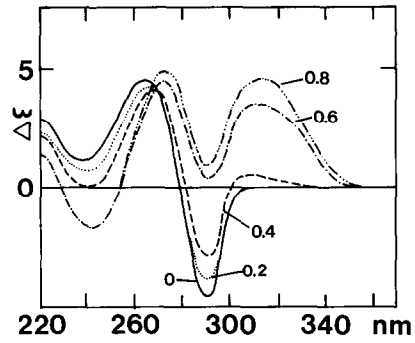

Fig.2. CD spectra of poly(dG–dC)·poly(dG–dC) in the Z-form in 1.5 mM CsCl, 60% ethanol with increasing amounts of Nt. The binding ratio r' is indicated.

CD band at 315 nm characteristic of the binding of Nt with a B-type structure is present. This suggests that a reversal from the Z to the B-type structure takes place under the action of Nt, even in the actual ionic and low water activity conditions that promotes the Z-form, due to the preferential interaction of Nt with the B-form of DNA. However, in contrast to Nt, Dst-3 does not induce any significant change in the CD spectrum, even at r' = 0.9. However, it should be noted that under low ionic conditions (such as 1 mM NaCl) where poly(dG–dC)·poly(dG–dC) exists as a B-type structure, Dst-3 interacts much better than Nt as judged from the intensity of the 320 nm band [28]. Increasing the ionic strength from 0.001–0.1 M nearly eliminates Dst-3 binding to B-form poly(dG–dC)·poly(dG–dC) (not shown).

On the other hand, Nt did not interact with poly(br^{8}dG–dC)·poly(br^{8}dG–dC) which is always in the Z form [29] and whose low salt CD spectrum is close to that of poly(dG–dC)·poly(dG–dC) in 60% ethanol (fig.2, r' = 0). No amount of Nt would reverse its spectrum to that of the B-form.

3.2. *Poly(dA–dC)·poly(dG–dT)*

In 60% ethanolic solution plus 1.5 mM CsCl and 0.02 mM Ca^{2+}, an intense, negative, long-wavelength CD band characterizes the non-B DNA conformation of this duplex [22]. Fig.3 shows the ability of Nt to interact with poly(dA–dC)·poly(dG–dT) in these conditions. Addition of Nt causes a gradual disappearance of the negative CD band at 278 nm; this effect is paralleled by the appearance of the positive CD band around 315 nm,

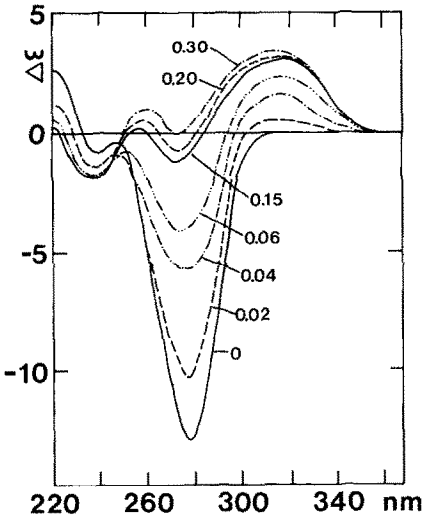

Fig.3. CD spectra of poly(dA–dC)·poly(dG–dT) in 1.5 mM CsCl, 60% ethanol, 0.02 mM Ca^{2+} with increasing amounts of Nt. Binding ratios r' are indicated.

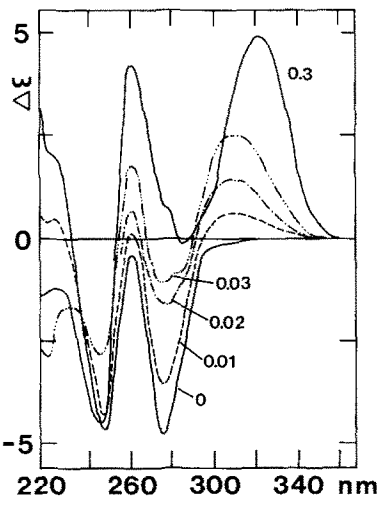

Fig.4. CD spectra of poly(dA–dT)·poly(dA–dT) in 1.5 mM CsCl, 60% ethanol, with increasing amounts of Nt. Binding ratios r' are indicated.

characteristic of the binding of Nt to the B-form. Contrary to poly(dG–dC)·poly(dG–dC), Dst-3 lowers also the negative band at 278 nm and induces the positive one at 320 nm; this requires, however, higher r' values than Nt. The ligand-induced conformational reversal is different in 6 M CsCl, a condition which also promotes the appearance of a non-B DNA conformation for poly(dA–dC)·poly(dG–dT) [22]. In 6 M CsCl, Nt does not interact with this duplex, in agreement with our findings that high [salt] dissociates Nt from poly(dA–dC)·poly(dG–dT) [30], whereas Dst-3 and Dst-5 show pronounced binding (not shown).

3.3. *Poly(dA–dT)·poly(dA–dT)*

The binding of Nt to this polymer in 60% ethanol is shown in fig.4. Under these conditions of decreased water activity this alternating duplex adopts also a modified conformation which is not a Z-type DNA and which differs from that at low ionic strength by an intense, negative, long-wavelength Cotton effect [24,25]. The progressive lowering of the negative CD band at 278 nm indicates that Nt reverses the low water form of poly(dA–dT)·poly(dA–dT) to the B-form as a consequence of its stabilizing effect on the B-conformation. This reversal takes place within a narrow range of ligand concentration up to $r' = 0.1$. In contrast with the two previous duplexes, Dst-3 is as efficient as Nt to induce the reversion to the B-form (CD spectra not shown).

4. DISCUSSION

Nt and Dst-3 are DNA binding ligands specific for the B-conformation [1–3]. These results show that these antibiotics can also reverse some alternating purine–pyrimidine duplexes to the B-form even under conditions of high [salt] and low water activity that induces a non-B form or the Z-form [18–22]. A reversal of the A to the B form has already been observed in DNA [11], and in some ribo-deoxy and 2′-fluoro-containing hybrids [12,13]. To compare easily the effects of Nt and Dst-3 on the 3 polymer duplexes studied one can examine the CD titrations monitored at two characteristic wavelengths (fig.5). The first positive CD peak at 315 and 328 nm for Nt and Dst-3, respectively, reflects the binding of the ligand to the duplex. The second wavelength corresponds to the negative peak around 275 to 288 nm which is characteristic for the Z form or at least of a non-standard B form [22–25] of poly(dA–dT)·poly(dA–dT) and poly(dA–dC)·poly-(dG–dT). This wavelength allows one to

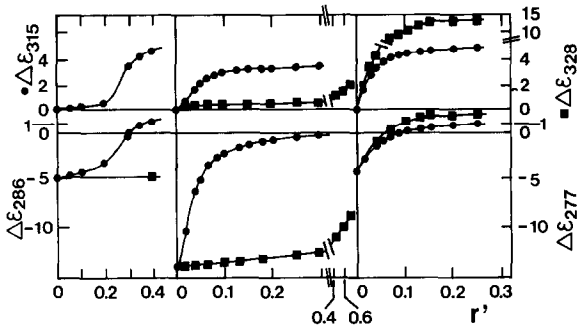

Fig.5. CD titrations of poly(dG–dC)·poly(dG–dC) (left), poly(dA–dC)·poly(dG–dT) (center) and poly(dA–dT)·poly(dA–dT) (right) with Nt (•) and Dst-3 (■). Experimental conditions are those of fig.2–4.

monitor the reversion of the non-B to the B-type CD spectrum (fig.5).

The following conclusions can be drawn:

(1) For a given antibiotic–polymer pair the titrations at the two wavelengths are always parallel. This strongly indicates that the binding of the antibiotic and the reversion to the B-form are concomitant and that essentially no free B-type polymer is present as an intermediate.

(2) An apparent inverse relationship exists between Nt binding and the ease of formation of non-B-like structures in alternating purine–pyrimidine duplexes. It is therefore not surprising that the easier a polymer binds Nt in low [salt], the more difficult it will be to turn it into the non-B forms and the more easily it will return to a B-form under the action of Nt.

(3) In the search for the mechanism of the reversion from the non-B like forms to the B forms by Nt one has to take into account the large differences in the action of Nt and Dst-3. The efficiency of Dst-3 to reverse non-B forms decreases from poly(dA–dT)·poly(dA–dT) to poly(dA–dC)·poly(dG–dT). Dst-3 is totally inefficient on poly(dG–dC)·poly(dG–dC) in the Z-form (60% ethanol, 1.5 mM CsCl), while it binds efficiently in 0.1 M NaCl to this polymer in the B-form.

Both Nt and Dst-3 are non-chiral molecules containing a plane of symmetry. Absence of binding of these molecules to the non-B-like or Z-form should therefore not be searched in the difference of handedness of such helices, but rather in the differences in their geometries. Nt binds to O^2 of pyrimidines or N^3 of purines in B-DNA, as well as to the phosphate sites [1–9]. The minor groove, where the distance between phosphate groups on opposite strands is ~12 Å, is most favourable for the interaction of both oligopeptides. In Z-DNA, however, the phosphate groups are much closer to each other [14–16,31] and N^3 of the purines is inaccessible because of the *syn* conformation of the guanosine residues, while the pyrimidine O^2 nearly touch the helix axis. The single groove of Z-DNA is very deep [14–16,31] so that interactions with phosphates and contacts with the bases are highly unfavoured. Nt thus induces a conversion to the B-form to which it binds with high efficiency.

It has been suggested [13] that Nt could replace one of the two water shells of the hydration spine in the small groove of the B-structure of A·T (and I·C) polymers [32]. The efficient reversal of poly(dA–dT)·poly(dA–dT) to the B-form by Nt and Dst-3 is probably accompanied by the replacement of the water spine by the antibiotics even under conditions of low water activity which do not favour the B-form. The absence of such a water spine in G·C pairs [32] greatly reduces binding, besides the steric hindrance by the 2-amino group of guanosine.

Our data also agree with recent calculations on different DNA conformations by the group of Pullman [33–35] which showed that the electrostatic potential is highest in the minor groove of B-DNA and represents an additional important factor in the binding ability of Nt and Dst-3. Interaction of both drugs with Z-DNA is electrostatically unfavoured [35].

In [21], reversal of the Z to B forms was observed under the action of intercalating drugs. Their finding that Dst-3 was ineffective on the Z-form of poly(dG–dC)·poly(dG–dC) agrees with these data in ethanolic solutions (fig.2,5).

Two points remain unclear and call for a deeper analysis of the Z to B reversal: the mechanism of the reversion by Nt and the inability of Dst-3 to reverse the Z-form of poly(dG–dC)·poly(dG–dC), although the latter antibiotic binds better to the B-form than Nt [28]. Work is under way to clarify these questions.

We have been informed recently by Professor C.W. Schmid (Univ. California, Davis) that poly(dA–dC)·poly(dG–dT) from Boehringer contained sequences different from those expected. This result, however, does not change our conclusion that a non-B DNA is returned to a B-type DNA under the influence of Nt and Dst-3. Whether this non-B form is in fact a Z-DNA (or any other poly(dA–dC)·poly(dG–dT) sample for that matter) will have to be resolved by other techniques and is under investigation.

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