

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

Christoph Zimmer, Christian Marck<sup>+</sup> and Wilhelm Guschlbauer<sup>+,\*</sup>

*Akademie der Wissenschaften der DDR, Zentralinstitut für Mikrobiologie und experimentelle Therapie, Abtlg. Molekulare Biochemie, Beutenbergstrasse 11, DDR-69 Jena, GDR and <sup>+</sup>Service de Biochimie, Département de Biologie, Centre d'Etudes Nucléaires de Saclay, F-91191 Gif-sur-Yvette Cedex, France*

Received 10 January 1983

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-type transition 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-

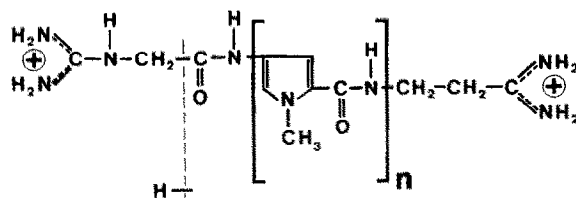


Fig.1. Chemical structure of netropsin (Nt;  $n = 2$ ) and distamycin-3 (Dst-3;  $n = 3$ ) lacking the left guanidinium group.

\* To whom correspondence should be addressed

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

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  $M^{-1} \cdot cm^{-1}$ ) [26]: Nt,  $\epsilon_{296} = 21\,500$ ; Dst-3,  $\epsilon_{303} = 33\,000$ . 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  $\epsilon_{254} = 8400$ ,  $\epsilon_{260} = 6500$  and  $\epsilon_{258} = 6800 M^{-1} \cdot cm^{-1}$ . 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

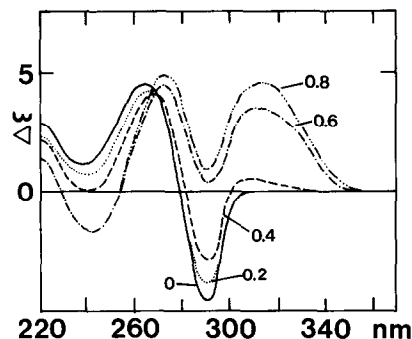


Fig.2. CD spectra of poly(dG–dC)·poly(dG–dC) in the Z-form in 1.5 mM CsCl, 60% ethanol with increasing amounts of Nt. The binding ratio  $r'$  is indicated.

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( $br^8dG$ –dC)·poly( $br^8dG$ –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,

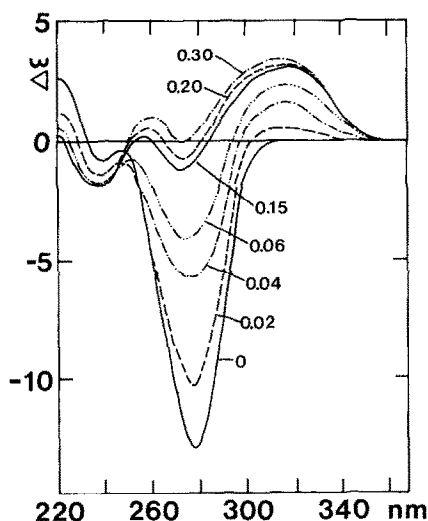


Fig.3. CD spectra of poly(dA-dC)·poly(dG-dT) in 1.5 mM CsCl, 60% ethanol, 0.02 mM  $\text{Ca}^{2+}$  with increasing amounts of Nt. Binding ratios  $r'$  are indicated.

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

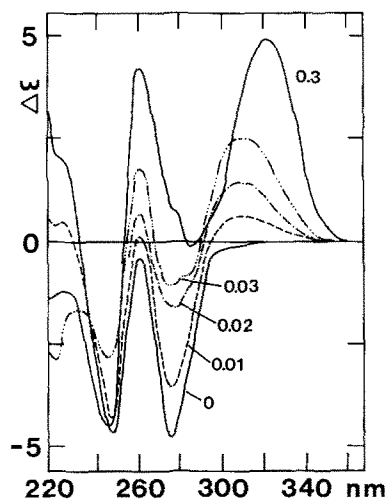


Fig.4. CD spectra of poly(dA-dT)·poly(dA-dT) in 1.5 mM CsCl, 60% ethanol, with increasing amounts of Nt. Binding ratios  $r'$  are indicated.

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

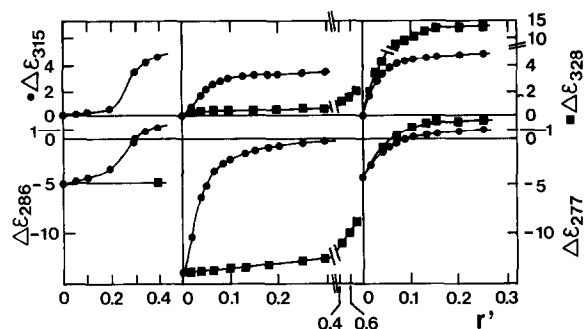


Fig.5. CD titrations of poly(dG-dC)·poly(dG-dC) (left), poly(dA-dC)·poly(dG-dT) (center) and poly(dA-dT)·poly(dA-dT) (right) with Nt (●) and Dst-3 (■). Experimental conditions are those of fig.2-4.

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<sup>2</sup> of pyrimidines or N<sup>3</sup> 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<sup>3</sup> of the purines is inaccessible because of the *syn* conformation of the guanosine residues, while the pyrimidine O<sup>2</sup> 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.

## REFERENCES

- [1] Zimmer, Ch. (1975) *Progr. Nucleic. Acids Res. Mol. Biol.* 15, 285-318.
- [2] Gursky, G.V. et al. (1977) in: *Nucleic Acid-Protein Recognition* (Vogel, H.J. ed) pp.189-217, Academic Press, New York.
- [3] Zimmer, Ch. (1983) *Commun. Mol. Cell. Biophys.*, in press.
- [4] Zimmer, Ch., Reinert, K.-E., Luck, G., Wähnert, U., Löber, G. and Thrum, H. (1971) *J. Mol. Biol.* 72, 329-348.
- [5] Luck, G., Triebel, H., Waring, M. and Zimmer, Ch. (1974) *Nucleic Acids Res.* 1, 503-530.
- [6] Zimmer, Ch., Luck, G., Burckhardt, G. and Lang, H. (1979) in: *Gene Function* (Rosenthal, S. ed) vol.51, pp.83-95, Pergamon, Oxford.
- [7] Luck, G. and Zimmer, Ch. (1973) *Stud. Biophys.* 40, 9-12.
- [8] Wartell, R.M., Larson, J.E. and Wells, R.D. (1974) *J. Biol. Chem.* 249, 6719-6731.
- [9] Zasedatelev, A.S., Gursky, G.V., Zimmer, Ch. and Thrum, H. (1974) *Mol. Biol. Rep.* 1, 337-342.
- [10] Ivanov, V.I., Minchenkova, L.E., Minyat, E.E., Frank-Kamenetskii, M.D. and Schyolkina, A.K. (1974) *J. Mol. Biol.* 87, 817-833.
- [11] Minchenkova, L.E. and Zimmer, Ch. (1980) *Biopolymers* 19, 823-831.
- [12] Zimmer, Ch., Kakiuchi, N. and Guschlbauer, W. (1982) *Nucleic Acids Res.* 10, 1721-1732.
- [13] Marck, Ch., Kakiuchi, N. and Guschlbauer, W. (1982) *Nucleic Acids Res.* 10, 6147-6161.
- [14] Wang, A.H.-J., Quigley, G.J., Kolpak, F.J., Crawford, J.L., Van Boom, J.H., Van der Marel, G. and Rich, A. (1979) *Nature* 282, 680-686.
- [15] Arnott, S., Chandrasekharan, R., Birdsall, D.L., Leslie, A.G.W. and Ratliff, R.L. (1980) *Nature* 283, 743-745.
- [16] Drew, H., Tanaka, T., Tanaka, S., Itakura, K. and Dickerson, R.E. (1980) *Nature* 286, 567-573.
- [17] Pohl, F.M. and Jovin, T.M. (1972) *J. Mol. Biol.* 67, 375-396.
- [18] Sage, E. and Leng, M. (1980) *Proc. Natl. Acad. Sci. USA* 77, 4597-4601.
- [19] Sage, E. and Leng, M. (1981) *Nucleic Acids Res.* 9, 1241-1250.
- [20] Ivanov, V.I. and Minyat, E.E. (1981) *Nucleic Acids Res.* 9, 4783-4798.
- [21] Van de Sande, J.H. and Jovin, T.M. (1982) *EMBO J.* 1, 115-120.
- [22] Zimmer, Ch., Tymen, S., Marck, Ch. and Guschlbauer, W. (1982) *Nucleic Acids Res.* 10, 1081-1091.
- [23] Vorličková, M., Kypr, J., Štokrová, S. and Šponar, J. (1982) *Nucleic Acids Res.* 10, 1071-1080.
- [24] Vorličková, M., Kypr, J., Kleinwächter, V. and Paleček, E. (1980) *Nucleic Acids Res.* 8, 3895-3913.
- [25] Vorličková, M., Sedláček, P., Kypr, J. and Šponar, J. (1982) *Nucleic Acids Res.* 10, 6969-6979.
- [26] Zimmer, Ch., Marck, Ch. and Guschlbauer, W. (1980) *Spectr. Lett.* 13, 543-554.
- [27] Wells, R.D., Larson, J.E., Grant, R.C., Shortle, B.E. and Cantor, C.R. (1970) *J. Mol. Biol.* 54, 456-497.
- [28] Luck, G., Zimmer, Ch., Reinert, K.H. and Arcamone, F. (1977) *Nucleic Acids Res.* 4, 2655-2670.
- [29] Lafer, E.M., Möller, A., Nordheim, A., Stollar, B.D. and Rich, A. (1981) *Proc. Natl. Acad. Sci. USA* 78, 3546-3550.
- [30] Zimmer, Ch., Marck, Ch., Schneider, Ch. and Guschlbauer, W. (1979) *Nucleic Acids Res.* 6, 2831-2837.
- [31] Crawford, J.L., Kolpak, F.J., Wang, A.H.-J., Quigley, G.J., Van Boom, J.H., Van der Marel, G. and Rich, A. (1980) *Proc. Natl. Acad. Sci. USA* 77, 4016-4020.
- [32] Drew, H.R. and Dickerson, R.E. (1981) *J. Mol. Biol.* 151, 535-556.
- [33] Pullman, B. and Pullman, A. (1981) *Stud. Biophys.* 86, 95-102.
- [34] Lavery, R. and Pullman, B. (1981) *Nucleic Acids Res.* 9, 7041-7051.
- [35] Lavery, R., Pullman, B. and Corbin, S. (1981) *Nucleic Acids Res.* 9, 6539-6552.