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# Specific interaction of netropsin, distamycin-3 and analogs with I.C duplexes: reversion towards the B form of the 2'-deoxy-.2'-deoxy-2'-fluoro- hybrid duplexes upon specific interaction with netropsin, distamycin-3 and analogs

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ABSTRACT: Binding of the B-form specific ligands netropsin and distamycin-3, -4 and -5 has been used to monitor the presence and/or the inducibility of a B-type structure in various poly-inosinic.poly-cytidilic double stranded polymers with deoxyribose, ribose or 2'-deoxy-2'-fluororibose as sugar on either strand. The efficiency of binding was followed by circular dichroism and further evaluated by the increase in melting temperature of the complexes. The efficient binding of netropsin and distamycins to the hybrid polymer $(dIfl)_n.(dC)_n$ demonstrated that the fluorine carrying strand may undergo a A to B-type transition reflecting a change of the 2'-deoxy-2'-fluororibose from the 3'-endo to the 1'-exo or 2'-endo pucker. The less efficient binding of the same ligands to the reverse hybrid $(dI)_n.(dCfl)_n$ showed that the geometry of the pyrimidine strand is the most critical for the specific interaction. Taking into account the recent findings about the regular hydration in the minor groove of the B-type dodecamer dCGCGAATTCGCG in solid-state, the different binding modes observed between the different polymers and antibiotics are explained by differences in their possibilities of hydration. Binding of netropsin to a double stranded deoxypolymer is interpreted as a local replacement of water molecules by netropsin in the minor groove hydration network which is typical of the B-form.

## INTRODUCTION

The functional and structural differences between RNA and DNA are mainly attributed to the difference in puckering of the ribose and deoxyribose rings (1). RNA can take only the A-form structure in which the ribose ring is 3'-endo puckered whereas DNA may adopt either the A-form, e.g. in low humidity fibres (1-3) and in ethanolic solutions (5,6) or more usually the B-form in which the deoxyribose is 2'-endo puckered (7). Among various suggestions (8-10), it has been proposed that the difference in electronegativity of the 2'-substituent -H and -OH, rather than their sizes, could determine the sugar puckering and thereby the helix structure type (10). Substitution in the 2' position of ribose by the strongly electronegative fluorine atom has been used for several years to check this hypothesis, although the fluorine atom is closer in size to the hydrogen than to the hydroxyl group (11-15). Substitution at the 2'-position by other highly electronegative substituents leads to an





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increased stability of the double stranded polymers (12,13,16-20).

In previous studies (20), it has been demonstrated that anti-$(rI)_n$.$(rC)_n$ antibodies are well recognized by fluoro-ribo I.C hybrids suggesting little or no alteration of the A-type duplex structure upon fluorine substitution on either one strand (20). On the other hand, it has been found that the substitution of the purine strand in $(dI)_n$.$(dC)_n$ by $(rI)_n$ and $(dIf1)_n$ results in duplexes retaining some features of the B-like helical structure (20).

In this paper, the conformational behaviour of I.C duplexes is further investigated with respect to their binding capacity for the DNA binding drugs netropsin (Nt), distamycin-3 (Dst-3) and the two analogs distamycin-4 (Dst-4) and distamycin-5 (Dst-5) (fig. 1). As demonstrated earlier, these drugs bind specifically in the minor groove of double stranded DNA's and A.T or I.C containing polymers by hydrogen bonding between the peptide groups and the 2-keto groups of the pyrimidines (21-30). These four antibiotics can therefore be used as specific conformational probes due to their exclusive affinity for the B-type double helix in solution (21-25). Suggestion for testing for the presence of a B-type inducible stucture in fluoro-deoxy hybrids came from the three crystalline structures of 2'-deoxy-2'-fluoronucleosides known so far. The pseudorotation angle ranges from P= 21° in dCf1 (31) to P= 29 and 41° in dIf1 (32) and up to P= 71° in dUf1 (31). This latter value is closed to the maximum of the energy barrier around P= 90° between the 3'-endo and 2'-endo states in ribonucleosides (33-35). The range from P= 21 to 71° therefore suggests that the energy barrier is displaced towards higher P values for the 2'-deoxy-2'-fluoronucleosides. On the other hand, the 3'-endo/2'-endo energy barrier which is partly due to the repulsion between the two neighbour hydroxyl groups in ribonucleosides could be lowered, in the crystal state, because of the stabilisation of the O(1')-endo conformation (P= 90°) by an internal hydrogen bond F(2') .... H-O(3') (31). The work described in this paper was undertaken to test the ability of the 2'-fluororibose ring to undergo the 3'-endo to 2'-endo transition in a fluoro-deoxy hybrid polymer. A ribo-deoxy hybrid duplex has

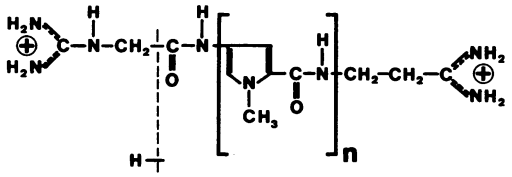

Figure 1: Chemical structure of netropsin (n=2). Distamycin-3, -4 and -5 are analogs lacking the left guanidinium group with n= 3, 4 and 5, respectively.

been recently shown to exist in fibre state as a distorded B-like helix with the bases slightly shifted out of the axis (36).

## MATERIAL AND METHODS

Polymers: $(dIfl)_n$ and $(dCfl)_n$ were synthesized as previously described (20). $(rI)_n.(rC)_n$ was from Choay, Paris and $(dI)_n.(dC)_n$ from Boehringer, Manheim (GFR). All double stranded hybrids were made by mixing the required homopolymers; $T_m$ were checked and found within 2 C of literature values (20,37-40). $(rI)_n$ and $(rC)_n$ were from Choay, $(dC)_n$ was from P.L. Laboratories, Milwaukee, WI.; $(dI)_n$ was isolated by preparative ultracentrifugation from $(dI)_n.(dC)_n$ (20). The concentrations of polymers used were from 0.6 to 1.4 $10^{-4}$ M/l phosphate. All CD measurements were made at 20 C, 0.1 M NaCl, 1 mM Tris pH= 8.50.

Antibiotics: Nt hydrochlorid was a gift from Drs. H. Thrum and Ch. Zimmer (Jena). Dst-3 was purchased from Boehringer, Dst-4 and Dst-5 were gifts from Dr. F. Arcamone (Milano). The following extinction coefficients were used: Nt $\varepsilon_{296}$= 21500, Dst-3 $\varepsilon_{303}$= 33000, Dst-4 $\varepsilon_{308}$= 39000, Dst-5 $\varepsilon_{316}$= 45000 (41). The ratio r' between drug concentration in the solution (free + bound) and phosphate concentration is used throughout this paper.

Instrumentation: UV absorbance spectra were recorded on a Zeiss DMR 10 spectrophotometer; $T_m$ measurements were recorded directly as previously described (20). CD spectra were recorded on Jobin-Yvon Dichrographes III and V.

## RESULTS

### Binding of Nt to $(dI)_n.(dC)_n$, $(dIfl)_n.(dC)_n$ and $(dI)_n.(dCfl)_n$.

The binding of Nt as well of Dst-3 to $(dI)_n.(dC)_n$ and $(dI\text{-}dC)_n.(dI\text{-}dC)_n$ has been described in previous reports (23,25). The CD spectrum of the complex $(dI)_n.(dC)_n$+Nt (together with those of the -Dst-3, -Dst-4 and -Dst-5 complexes) is shown in fig. 2. The interaction spectrum displays two positive CD bands (315 and 247 nm) and two negative bands (272 and 225 nm) (fig. 2,a) as do those of other double stranded A.T-containing polymers and DNA's. The binding of Nt to the hybrid polymer $(dIfl)_n.(dC)_n$ is nearly as efficient as to $(dI)_n.(dC)_n$ (fig. 3). The CD signal at saturation of Nt binding gives $\Delta\varepsilon$= 3.5 for the $(dI)_n.(dC)_n$ complex (fig. 5) and $\Delta\varepsilon$= 3.2 for the $(dIfl)_n.(dC)_n$ complex (fig. 6). The interaction spectrum of the $(dIfl)_n.(dC)_n$ complex lacks the second positive maximum at 247 nm when compared with the $(dI)_n.(dC)_n$ complex, but it should be noted that the spectra of these two complexes at saturating Nt level are closer to each other than those of the free polymers (the CD 245/267 ratio are -1.2 and -15.7 for free $(dI)_n.(dC)_n$ and $(dIfl)_n.(dC)_n$, res-

pectively, while for the complexes these ratios become -1.2 and -1.5). This may reflect some alteration in the double helix geometry of the $(dIfl)_n.(dC)_n$ complex which requires some flexibility to adopt a B-type stucture as it exists in the $(dI)_n.(dC)_n$ complex. On the other hand, the deviation of the interaction spectra around 247 nm may be indicative of subtle differences in the conformation of the isohelicaly oriented oligopeptide since the induced CD (interaction spectrum) reflects the conformatioaal properties of the bound ligand (24,26,30).

The binding efficiency of Nt to the reverse hybrid $(dI)_n.(dCfl)_n$ is, however, much lower compared to $(dIfl)_n.(dC)_n$; the CD spectrum of the $(dI)_n.(dCfl)_n$+Nt complex is shown in fig. 4 and the binding curve in fig. 7. While the CD signal at 315 nm is similar for the Nt complexes of $(dI)_n.(dC)_n$ and $(dIfl)_n.(dC)_n$ ($\Delta\varepsilon$= 3.5), the corresponding value is only $\Delta\varepsilon$= 1.5 in the case of the (dI) .(dCfl) complex and much higher concentrations of Nt are needed to reach saturation (around 1.0 Nt/P).

Binding of Dst-3, Dst-4 and Dst-5 to $(dI)_n.(dC)_n$.

Unlike Nt, all three distamycins bind in a two-step mechanism as reflected by the displacement of the isodichroic points observed in the titration experi-

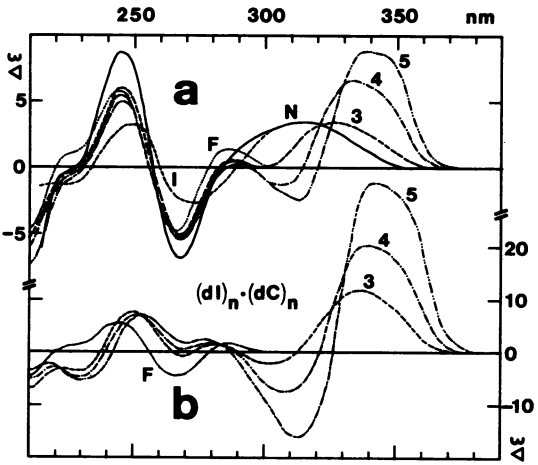


Figure 2: CD spectra of $(dI)_n.(dC)_n$ complexed with the different antibiotics at 20 C, 0.1 M NaCl, 1 mM Tris pH= 8.5: (a) F (·····) free duplex; N (————) Nt complex at r'= 0.1 (saturation); I (– – – – –) interaction spectrum (N-F) of the Nt complex at r'= 0.1; 3 (— — — —) Dst-3 complex at r'= 0.033 (first titration step); 4 (—·—·—) Dst-4 complex at r'= 0.033; 5 (—··—··—) Dst-5 complex at r'= 0.033 (b) 3 (— — — —) Dst-3 complex at r'= 0.23 (second titration step); 4 (—·—·—) Dst-4 complex at r'= 0.23; 5 (—··—··—) Dst-5 complex at r'= 0.23.

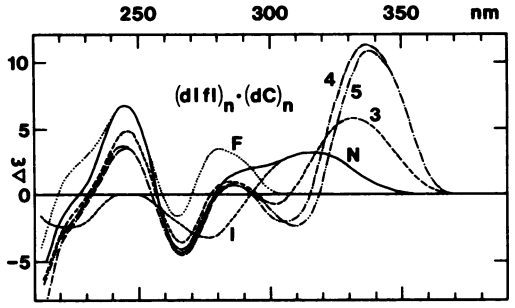


Figure 3: CD spectra of $(dIfl)_n.(dC)_n$ complexed with the different antibiotics. Same conditions as in fig. 2: (a) F (·····) free duplex; N (————) Nt complex at r'= 0.14 (saturation); I (– – – – –) interaction spectrum (N-F) of the Nt complex at r'= 0.14; 3 (— — — —) Dst-3 complex at r'= 0.18 (saturation); 4 (—·—·—) Dst-4 complex at r'= 0.15; 5 (—··—··—) Dst-5 complex at r'= 0.12.

ments (see table I). As an example, the complete titration of $(dI)_n.(dC)_n$ by Dst-3 is shown in fig. 9. The CD spectra of the three $(dI)_n.(dC)_n$ complexes before the end of the first step are shown in fig. 2,a (r'= 0.03). During this

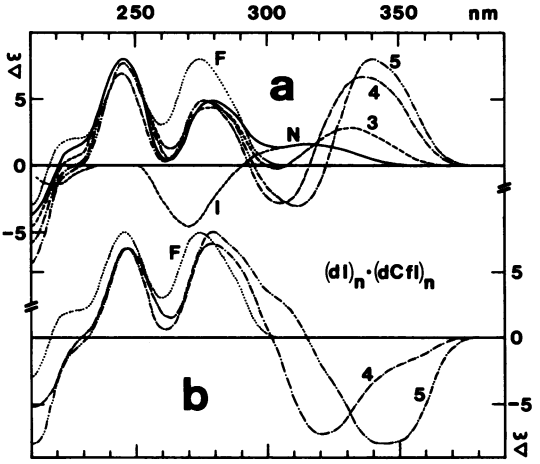


Figure 4: CD spectra of $(dI)_n.(dCfl)_n$ complexed with the different antibiotics. Same conditions as in fig. 2: (a) F (·····) free duplex; N (————) Nt complex at r'= 0.9 (saturation); I (– – – – –) interaction spectrum (N-F) of the Nt complex at r'= 0.9: 3 (— — — —) Dst-3 complex at r'= 0.8 (saturation); 4 (—·—·—) Dst-4 complex at r'= 0.1 (first titration step); 5 (—··—··—) Dst-5 complex at r'= 0.08; (b) 4 (—·—·—) Dst-4 complex at r'= 0.6 (saturation); 5 (—··—··—) Dst-5 complex at r'= 0.5

first binding step, Dst-3 and analogs behave similarly to Nt upon binding to $(dI)_n.(dC)_n$ as judged from the CD spectra of the complexes (fig. 2,a). The positive maximum in the long wavelength range of the interaction spectrum shifts from 327 to 333 and 337 nm with increasing number of methylpyrrole-carboxamide units when going from Dst-3 to Dst-4 and Dst-5, respectively. This effect is typical of this series of drugs upon binding to B-DNA's (22,44). The second binding mechanism of Dst-3, -4 and -5 is characterized by a progressive red shift of the first CD band and a displacement of the isodichroic points to higher wavelengths (fig. 2 and 9, table I). The two-step binding mechanism can be conveniently monitored at 267 nm (fig. 5,b). The r' values for the change from the first to the second type of binding mechanism (change in the isodichroic points wavelengths) can be evaluated in the vicinity of 0.060, 0.052 and 0.043 for Dst-3, -4 and -5, respectively. These values correspond to 8.3, 9.6 and 11.6 base pairs per antibiotic molecule suggesting that proportionaly fewer Dst-5 molecules are required to initiate the second type of interaction due to the larger size of Dst-5 relative to Dst-3. The titration curve of the $(dI)_n.(dC)_n$+Dst-5 complex monitored at the first positive CD maximum

Table I: Isodichroic points wavelengths (nm) observed during the titrations of $(dI)_n.(dC)_n$, $(dIfl)_n.(dC)_n$ and $(dI)_n.(dCfl)_n$ by Nt, Dst-3, Dst-4 and Dst-5 in 0.1 M NaCl, 1 mM Tris, pH= 8.5.

| | | | | | |
|---|---|---|---|---|---|
| $(dI)_n.(dC)_n$ | + Nt | | 233 | 259 | 289 |
| " | + Dst-3 | 1st step | 242.5 | 263 | 300 |
| | | 2nd step | 245 | 291 | 326 |
| " | + Dst-4 | 1st step | 250 | 261 | 314 |
| | | 2nd step | 247 | 285 | 323 |
| " | + Dst-5 | 1st step | 250 | 251 | 320 |
| | | 2nd step | 249 | 285 | 326 |
| $(dIfl)_n.(dC)_n$ | + Nt | | 240 | 245 | 293 |
| " | + Dst-3 | | - | - | 307 |
| " | + Dst-4 | | - | - | 314 |
| " | + Dst-5 | | - | - | 319 |
| $(dI)_n.(dCfl)_n$ | + Nt | | 235 | 252 | 292 |
| " | + Dst-3 | | 240 | 255 | 307.5 |
| " | + Dst-4 | 1st step | - | - | 316 |
| | | 2nd step | 245 | 255 | 307 |
| " | + Dst-5 | 1st step | - | - | 322 |
| | | 2nd step | 253 | 258 | 319 |

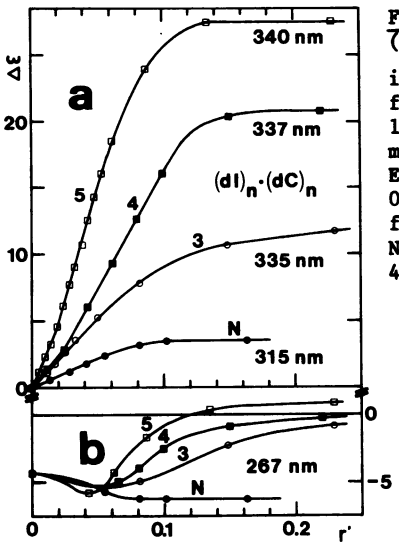


Figure 5: Binding curves for $\overline{(dI)_n.(dC)_n}$ as a function of increasing amounts of the different antibiotics at the wave lengths indicated. Same experimental conditions as in fig. 2. Experimental points below r'= 0.05 in (b) have been omitted for clarity.
N (●): Nt; 3 (○): Dst-3; 4 (■) : Dst-4; 5 (□): Dst-5.

outside the spectral range of the polymer (fig. 5,a) shows a kink at r'= 0.02 (this value, however, does not correspond to the end of the first step r'= 0.043). Such an effect has been previously observed for the complex $(dA\text{-}dT)_n.(dA\text{-}dT)_n$+Dst-5 and this could reflect some subtle conformational changes during the initial interaction which possibly facilitate the binding of the successive molecules (45).

Binding of Dst-3, Dst-4 and Dst-5 to $(dIfl)_n.(dC)_n$ and $(dI)_n.(dCfl)_n$.

As found for Nt, Dst-3 and analogs bind efficiently to $(dIfl)_n.(dC)_n$; however, in contrast to $(dI)_n.(dC)_n$, $(dIfl)_n.(dC)_n$ binds Dst-3, -4 and -5 in a single step mechanism (fig. 3). The substitution in the purine strand by the fluorine at the 2' position thus eliminates the possibility of a second binding mechanism. Examination of the CD spectra does not permit us to identify unambiguously the unique step of the $(dIfl)_n.(dC)_n$+Dst-3 (-4,-5) titration with either of the two steps of the $(dI)_n.(dC)_n$+Dst-3(-4,-5) titration.

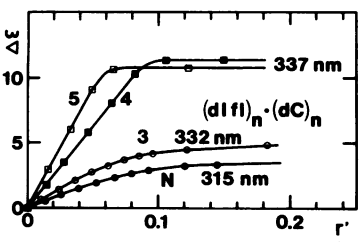


Figure 6: Binding curves for $\overline{(dIfl)_n.(dC)_n}$ as a function of increasing amounts of the different antibiotics at the wavelengths indicated. Same experimental conditions as in fig. 2. Same symbols as in fig. 5.

Results on binding of Dst-3, -4 and -5 to the reverse hybrid $(dI)_n.(dCfl)_n$ again show different peculiarities in the interaction mode, compared with $(dIfl)_n.(dC)_n$. First, the hybrid containing $(dCfl)_n$ exhibits less binding efficiency for Dst-3, which is similar to the Nt interaction (fig. 4 and 7); secondly, the two longest antibiotics Dst-4 and -5 bind more efficiently but exhibit a two-step, however different binding mode (fig. 4a. and b). This second binding mechanism is characterized by a large decrease of the CD binding signal at longer wavelength around 337 and 340 nm for Dst-4 and -5, respectively. In both cases, the initial positive CD band turns into a negative one (up to $\Delta\varepsilon$= -7 at high r' values), an effect not previously observed in CD binding studies for other DNA's. The inversion of the CD interaction signal starts at one oligopeptide molecule per 4.5 base pairs for Dst-4 and at 5.6 base pairs for Dst-5 (fig. 7). This inversion of the long wavelength CD band suggests that the second binding mode found for $(dIfl)_n.(dC)_n$ is different from the second binding mode observed during the binding of Dst-3; -4 and -5 to $(dI)_n.(dC)_n$.

Binding of Dst-4 and Dst-5 to $(rI)_n.(dC)_n$ and $(dI)_n.(rC)_n$.

Nt does not interact with $(rI)_n.(dC)_n$, in 0.1 M NaCl whereas Dst-3 shows weak CD binding effects (not shown). With increasing chain length of the oligopeptide system, such as in Dst-4 and -5, binding occurs to $(rI)_n.(dC)_n$ in a two-step mechanism caracterised by an inversion of the positive CD band around 340 nm (fig. 8). The behaviour of the reverse hybrid $(dI)_n.(rC)_n$ is quite similar in its binding tendency for the same oligopeptides. In fig. 8 are shown the binding curves of Dst-4 and -5 to both ribo-deoxy hybrids followed at two wavelengths: 340 nm (the first positive maximum) and 245 nm. This latter wavelength is useful to follow the I.C interaction. At 245 nm all four of the single stranded polymers $(dI)_n$, $(dC)_n$, $(rI)_n$ and $(rC)_n$ display negative or weak positive CD signal (37,39). A decrease of the CD signal at this wavelength

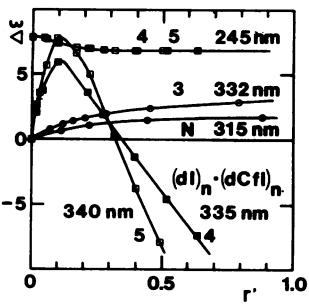


Figure 7: Binding curves for $(dI)_n.(dCfl)_n$ as a function of increasing amounts of the different antibiotics at the wavelengths indicated. Same experimental conditions as in fig. 2. Same symbols as in fig. 5. Note the change in r' scale with respect to figs. 5 and 6.

occuring in any I.C double stranded polymer antibiotic complex thus indicates a loss of the double helix structure. Both $(rI)_n.(dC)_n$ and $(dI)_n.(rC)_n$ duplexes display such a lowering of the CD signal at 245 nm upon Dst-5 binding (fig. 8). This should be compared with the behaviour of $(dI)_n.(dCfl)_n$ upon binding of Dst-4 and -5 (fig. 7). For $(dI)_n.(dCfl)_n$, the increase of negative CD signal at 340 nm is paralleled by a very weak loss of the CD amplitude at 245 nm. This suggests that, contrary to $(rI)_n.(dC)_n$ and $(dI)_n.(rC)_n$, $(dI)_n.(dCfl)_n$ is resistant to the strand separation induced by Dst-4 and -5. This helix destabilizing mechanism appears as a consequence of an incompatibility between the geometry of the ribo- strand (A-type) and the geometry imposed on the deoxy- strand by the antibiotic (B-type). It should be noted also that $(dI)_n.(rC)_n$ is the least stable of the nine I.C duplexes (20), having a $T_m$ of 35 C under the present conditions while $(dI)_n.(dCfl)_n$ has a $T_m$ = 48 C.

Thermal stabilization of the duplexes by Nt, Dst-3, Dst-4 and Dst-5 binding.

The binding of Nt stabilizes the double stranded structure of DNA's and synthetic duplexes and increases the melting temperature (25,27,42,43). Below Nt saturation (r'= 0.1 Nt/P), a biphasic melting curve is found for the $(dA-dT)_n.(dA-dT)_n$+Nt complex (27,42). A similar clear-cut biphasic curve is also observed during the melting of the $(dI)_n.(dC)_n$+Nt complex while a single monophasic melting is again found at r'= 0.1 Nt/P and higher concentrations. The largest stabilizing effect observed among the five duplexes studied is found for $(dI)_n.(dC)_n$ (see table II). Due to the increasing number of hydrogen bonds from Dst-3 to Dst-5 (4 to 6), the stabilization increases parallely; Nt stabilization being however more efficient than that of Dst-3 and Dst-4. For all four $(dI)_n.(dC)_n$+antibitotic complexes studied, $T_m$ increases significantly even beyond saturating level. The stabilization of $(dIfl)_n.(dC)_n$ is much less efficient and Dst-3 stabilizes also less than Nt. The stabilization of $(dI)_n.(dCfl)_n$ is even weaker and very small or undetectable for the ribo-deoxy hybrids. Moreover, as Dst-5 concentration increases, the thermal hyperchromi-

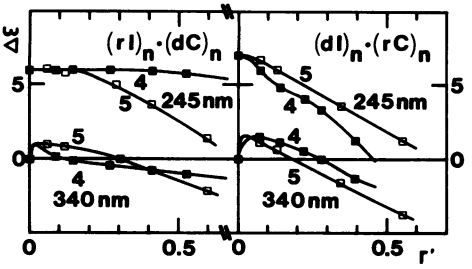


Figure 8: Binding curves for $(rI)_n.(dC)_n$ and $(dI)_n.(rC)_n$ as a function of increasing amounts of Dst-4 and Dst-5 at the wavelengths indicated. Same experimental conditions as in fig. 2.
4 (■): Dst-4;
5 (□): Dst-5.

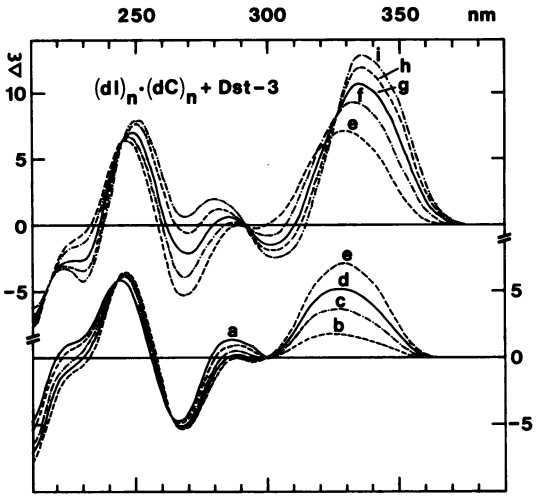


Figure 9: CD spectra of $(dI)_n.(dC)_n$ in the presence of increasing concentration of Dst-3: (a) first step of titration: a (————) r'= 0.0; b (— — — —) r'= 0.017; c (—·—·—·—) r'= 0.033; d (————) r'= 0.049; e (— — — —) r'= 0.66; (b) second step of titration: e (— — — —) r'= 0.066; f (—·—·—·—) r'= 0.11: g (————) r'= 0.15;; h (— — — —) r'= 0.23; i (—·—·—·—) r'= 0.40.

city of the ribo-deoxy hybrids+Dst-5 complexes decreases and for r'= 0.8 Nt/P no more melting is observed. Both ribo-deoxy hybrids exhibit negative CD signals around 340 nm accompanied by a lowering of the CD at 245 nm. $T_m$ experiments therefore show that these CD changes correspond to a destruction of the double helix.

Table II: Increase of melting temperature ($\Delta T_m$) upon Nt, Dst-3, Dst-4 and Dst-5 binding at 0.1 M NaCl, 1 mM Tris, pH= 8.5.

| | $T_m$(C) | r' | $\Delta T_m$(C) | | | |
|---|---|---|---|---|---|---|
| | | | Nt | Dst-3 | Dst-4 | Dst-5 |
| $(dI)_n.(dC)_n$ | 46 | 0.1 | 26.6 | 18.2 | 24.3 | 29.6 |
| | | 0.2 | 29.6 | 20.8 | 26.5 | 31.1 |
| | | 0.4 | 31.9 | 23.6 | 28.9 | 33.0 |
| | | 0.8 | 32.7 | 26.9 | 30.8 | 35.6 |
| $(dIfl)_n.(dC)_n$ | 61 | 0.2 | 3.9 | 2.1 | 5.5 | 10.4 |
| $(dI)_n.(dCfl)_n$ | 48 | 0.2 | 1.1 | 1.7 | 5.0 | 10.6 |
| $(rI)_n.(dC)_n$ | 52 | 0.2 | 0. | 0. | 0. | 1. |
| $(dI)_n.(rC)_n$ | 35 | 0.2 | 1. | 1.6 | 1.9 | 2.3 |

## DISCUSSION

The exclusive affinity of Nt for the B-type conformation allows to monitor the presence or the inducibility of this conformation in a double-stranded polynucleotide. In contrast to $(dI)_n.(dC)_n$ which is in the B-form, both ribo-deoxy hybrids $(rI)_n.(dC)_n$ and $(dI)_n.(rC)_n$ do not interact with Nt. Therefore, in accordance with the data on the geometry and stability of these duplexes (37,46,47), the lack of any specific binding suggests the impossibility of conversion from the A-type into the B-type conformation under the action of Nt. Dst-4 and Dst-5 display some low binding affinity for these hybrids as judged from the CD interaction spectra but this specific binding is rapidly followed by a non-specific interaction (fig. 8).

The behaviour of the fluoro-deoxy hybrids is clearly different from that of the ribo-deoxy hybrids. $(dIf1)_n.(dC)_n$ interacts with Nt nearly as well as does $(dI)_n.(dC)_n$ (fig. 2 and 3) ; it therefore appears that this hybrid is less restricted in its conformational flexibility than the ribo-deoxy hybrids. This agrees with recent data on Nt binding to the analog hybrid polymers $(dAf1)_n.(dT)_n$ and $(dAf1)_n.(dU)_n$ (48). The reverse hybrid, $(dI)_n.(dCf1)_n$ , carrying the 2'-fluororibose in the pyrimidine strand exhibits much less binding capacity (fig. 4), indicating that this hybrid is more resistant to structural changes towards the B-form. From the binding of Nt to the two fluoro-deoxy hybrids, two main conclusions can be drawn:

(i) Substitution by a fluorine atom in the 2' position forces the ribose pucker to the 3'-endo configuration and consequently favours the A conformation in a hybrid duplex. The geometry of a fluoro-deoxy hybrid duplex is, however not restricted and a higher flexibility compared with the ribo-deoxy hybrids allows a change of the 2'-fluororibose pucker to the B-form typical 1'-exo or 2'-endo conformations upon binding of Nt.

(ii) When comparing both reverse hybrids $(dIf1)_n.(dC)_n$ and $(dI)_n.(dCf1)_n$, binding of Nt appears more efficient when the purine strand bears the fluorine substitution. This is not surprising since it is the pyrimidine strand that is recognized by Nt through hydrogen bonding. The CD spectrum of $(dIf1)_n.(dC)_n$ is also much closer to that of of $(dI)_n.(dC)_n$ than is that of $(dI)_n.(dCf1)_n$ (fig. 2-4). This shows that the geometry of $(dIf1)_n.(dC)_n$ is closer to that of $(dI)_n.(dC)_n$ than is that of $(dI)_n.(dCf1)_n$.

In a previous study, it has been shown that the replacement of a ribo-strand by a fluoro- strand always enhances the "ribo-" character of a duplex: higher $T_m$, A-like conformation, antigenicity, interferon inducing (20). The efficient binding of the B-form specific ligands Nt and Dst-3, -4 and -5 to

$(dIfl)_n.(dC)_n$ and to a lesser extent to $(dI)_n.(dCfl)_n$ is an unexpected result on the basis of the previous data on these polymers. The more stable A-form of the fluoropolymers is possible in spite of the smaller size of the fluorine atom with respect to 2' hydroxyl group which is both much more electronegative and larger than hydrogen. The main reason why RNA does not take the B-form structure while a fluoropolymer may do so upon binding of Nt therefore appears to be that 2' hydroxyl would produce both steric and electrostatic repulsions between adjacent residues in B-form (36,49). The geometry of the 2' substituent in the A and B forms can be summarized as follows. In the A-form helix the mean plane of the sugar lies on the surface of the cylinder defined by the helix with the 2' substituent protruding radially on the edge of the minor groove. After transition to the B-form, the sugar is turned 90° to become radial and the 2' substituent has to face a cage like cavity formed by O(5'), O(1') and H bound to C(6)-pyr or C(8)-pur of the next unit in the 3' direction. CPK model building shows that the B-type helix cannot accomodate a 2' hydroxyl group unless considerable steric hindrance with these three atoms (36). The ability of a fluoropolymer to undergo the A to B transition upon Nt binding therefore appears to be possible because of the smaller size of the fluorine in spite of its intrinsic tendency to prefer the 3'-endo puckering. In solution, the 2'-fluoronucleosides show a greater preference for the 3'-endo state than do the ribonucleosides as determined by NMR measurements (49-53). In certain cases, the 2'-fluoronucleosides may behave like deoxynucleosides : dUflMP is a pseudo-substrate of thymidylate synthetase whose natural substrate is dUMP (14,15). The crystal structure of dUfl exhibits the unusual puckering 4'-exo, O'-endo (32), such a configuration is mid-way between the 3'-endo and 1'-exo configurations, typical of ribo - and deoxynucleosides, respectively. Theoretical computations predict a lower energy barrier between the 3'- and 2'-endo states for 2'-fluoro- than for ribonucleosides (35). On the other hand, the internal hydrogen bond type interaction occuring in the solid state for monomers (31) cannot hold for the polymers. The question of how much the lowering of the 3'/2'-endo energy barrier facilitates the A to B transition in the fluoropolymers remains opened.

Recent data on B-DNA crystal structure have shown that the minor groove is occupied by a regular water network in the AT clusters (54-58). Upon binding to DNA, Nt has to displace these water molecules to bind on O2-pyr and/or N3-pyr. Nt would thus not be considered as binding to the classical B-DNA structure (i.e. non-hydrated), requiring some, although not extensive,

alteration of the B double helix itself . On the contrary, one may consider the association of Nt with DNA as a local replacement of the minor groove structure by a molecule of Nt that becomes part of the B-type structure itself, its geometry being ideally suited to mimic the two deepest water shells (57). In this model of Nt lying along the minor groove, first proposed by Berman et al.(59), the two positively charged end groups would lie equally spaced from both phosphate chains similarly to the charged group of daunomycin when complexed to dCGTACG (60). The difference in hydration are probably responsible for the different mechanisms of antibiotic binding encountered. Dst-3, -4 and -5 bind to $(dI)_n.(dC)_n$, as well as to $(dA)_n.(dT)_n$ (not shown), in a two-step mechanism while Nt binds in a single step. It could be possible that the first step of Dst-3; -4 and -5 binding to the above polymers represents the binding to a still partially hydrated structure. On the other hand, the unique step observed in the binding of Dst-3, -4 and -5 to $(dIf1)_n.(dC)_n$ could be due to the fact that this polymer lacks the minor groove water network due to the A-form imposed by the fluoro strand as found in the A-form solid-state structures of $d^{I}CCGG$ and dGGTATACC (61,62).

The results presented here show that the substitution by fluorine in the 2' position of deoxyribose in either strand confers on DNA two somewhat conflicting properties: on one hand higher thermal stability which is typical of ribopolymers and, on the other hand, Nt and Dst specific binding ability which is typical of deoxypolymers.

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