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Interaction of synthetic analogs of distamycin with DNA

Role of the conjugated *N*-methylpyrrole system in specificity of binding

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Interaction of two synthetic analogs of distamycin (Dst), PPA and PAP, containing a saturated β-alanine moiety replacing one *N*-methylpyrrole ring, with different polynucleotides and natural DNAs were studied using UV and CD spectroscopy. The results indicate that, similar to Dst, these analogs bind to DNA via the minor groove with a specificity towards AT-base pairs. It may be proposed that pyrrole chromophores in Dst probably do not play a role in the AT-base selectivity exhibited by Dst.

Drug-DNA interaction; Distamycin analog; AT-base specificity; Minor groove binding

1. INTRODUCTION

Distamycin (Dst) and netropsin (Nt) represent a class of ligands which bind to DNA via the minor groove, in a nonintercalative fashion. The characteristic AT-base specific nature of the binding of these ligands has been ascribed to electrostatic, van der Waals and hydrogen bonding interactions [1,2]. These pyrrole-amide compounds are potent antibiotics. They are cytotoxic too, and this limits their therapeutic uses [1]. In our laboratory, we have recently initiated studies on the DNA-binding characteristics and antibiotic properties of certain synthetic analogs of Dst and Nt with a view to (i) understanding the conformational and chemical basis of the binding specificity, which is not yet fully explained, and (ii) designing drugs having similar biological activities as Dst/Nt but lower cytotoxicity. Here we wish to report the DNA-binding characteristics of two analogs of Dst (structures shown in fig.1). The distinctive feature of these analogs, β-[1-methyl-4-(1-methyl-4-formylaminopyrrole-2-carboxamido)-2-carboxamido]-propionamidopropionamidine (abbreviated PPA) and 1-methyl-4-[β-(1-methyl-4-formylaminopyrrole-2-carboxamido)propionamido]pyrrole-2-carboxamidopropionamidine (abbreviated PAP), is the disruption of the structural repetition of *N*-methylpyrrole units. The objective of the present study is to examine the role of extensive delocalisation of the electron density (via the presence of consecutive *N*-methylpyrrole carboxamide units) upon the specificity of binding exhibited by Dst and Nt. The replacement of the *N*-methylpyrrole rings by the saturated β-alanine moiety neither significantly alters the backbone curvature of the ligands, nor does it apparently change the distances between the potential hydrogen bonding sites in the amide residues. Thus, these analogs represent good model systems to study the role of the *N*-methylpyrrole rings in Dst-DNA interaction. The results of the present communication suggest that both PPA and PAP bind to B-DNA via the minor groove with preference for AT-base pairs.

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Abbreviations: poly(dA-dT), alternating copolymer poly(dA-dT)·poly(dA-dT); poly(dG-dC), alternating copolymer poly(dG-dC)·poly(dG-dC)

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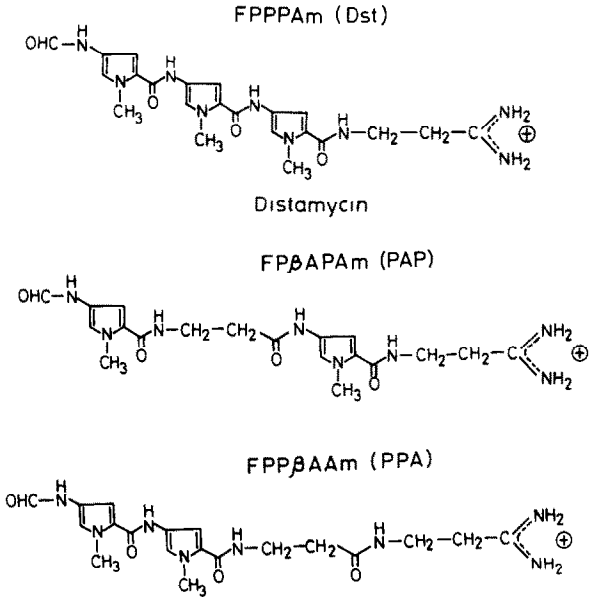

Fig.1. Structural formulae for Dst, PPA and PAP. The code names FPPPAm etc. denote the constituents: F, formyl group; P, *N*-methylpyrroleamide; βA, β-alanine; Am, amidine.

The significance of the present observation vis-a-vis Dst-DNA interaction is discussed in the conclusion.

2. MATERIALS AND METHODS

PPA and PAP were synthesised (as hydrochloride salts) by a modification of the procedure [3] for the total synthesis of Dst. The synthetic products were identified by IR, NMR and mass spectroscopic data (unpublished). Poly(dA-dT) and poly(dG-dC) (from Pharmacia P.L.) were used without further purification. The natural DNAs (from Sigma) were deproteinated by phenol extraction and extensively dialysed against 20 mM NaCl, pH 7.1, before use. The concentrations of PPA and PAP were determined from their extinction coefficients (in $mM^{-1} \cdot cm^{-1}$) $\epsilon_{293} = 15.4$ and $\epsilon_{275} = 11.4$, respectively. UV and CD spectroscopic measurements were done with a Beckman DU-8B spectrophotometer and a Jasco J-20C spectropolarimeter (with a Jasco DP-500N data processor), respectively. All measurements were carried out in 20 mM NaCl (pH 7.1) at 20°C. Circular dichroic values are expressed as molar ellipticity, $[\theta]$.

3. RESULTS AND DISCUSSION

3.1. *Binding with DNA*

The binding of these ligands to DNA is indicated from the change in the UV absorption spectra of the ligands in the presence of poly(dA-dT). Representative examples of the spectra are shown in fig.2. Similar to what was observed for Dst in the presence of DNA [1], a red shift of the long wavelength peak of the ligands, PPA and PAP, ensues their binding to DNA. Inspection of the spectra of PPA and PAP in the presence of other DNAs (not shown) suggests that the extent of the red shift depends on the nature of the DNA, e.g., for both PPA and PAP, the extent of the red shift follows the order poly(dA-dT) > calf thymus (c.t.) DNA > poly(dG-dC). It is noted from fig.2 (and also from the spectra of PPA and PAP in the presence of other DNAs) that the spectrum of PPA exhibits a longer red shift in the presence of a DNA than the spectrum of PAP does.

The binding of these ligands to DNA is further confirmed from the observation of the induced CD bands in the 290–350 nm region of the CD spectra of the ligand-DNA complexes (fig.3). Similar induced CD bands (but with higher molar ellipticity values) characterize the association of Dst with synthetic and natural DNA duplexes [4]. The spectra of the free polymers are also shown for comparison. Notable features of the CD spectra shown in fig.3 can be summarized as: (i) there is no significant change in the conservative B-DNA type CD spectra due to binding, (ii) the induced CD band for PPA-DNA complex is more red-shifted

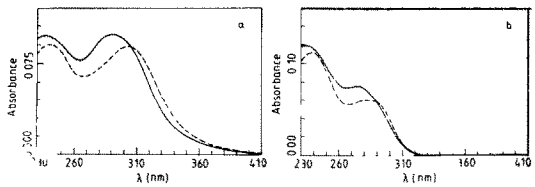

Fig.2. UV absorption spectra of (a) PPA (——) (7 μM) and (b) PAP (——) (7 μM) and their complexes (– – –) with poly(dA-dT), for $r' = 0.08$. Spectra of the complexes were recorded against the blank containing an equal concentration of poly(dA-dT).

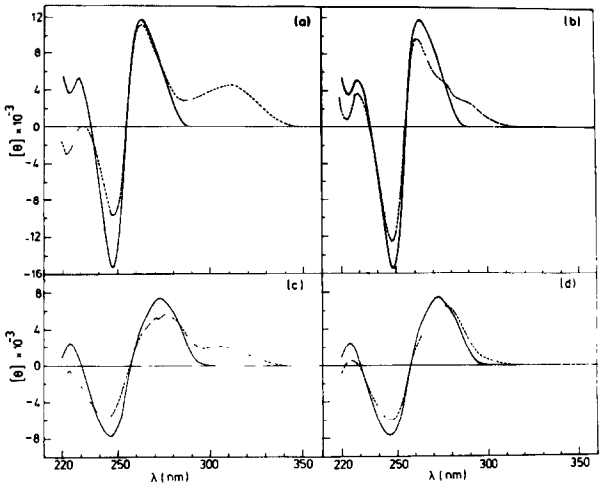

Fig.3. CD spectra of the free poly(dA-dT) (300 μM) (——) and complexes (– – –) with (a) PPA and (b) PAP; CD spectra of free c.t. DNA (350 μM) (——) and complexes (– – –) with (c) PPA and (d) PAP. In all cases for the complexes, $r' = 0.09$.

and has a higher ellipticity value compared to that for the PAP-DNA interaction. For the PAP-c.t. DNA complex there is no definite peak in the induced band.

3.2. *Base specificity of binding*

UV and CD spectroscopic studies on the association of these ligands with DNA also enabled us to determine the base-specific nature of the binding: (i) the change in absorbance of the ligand upon addition of the polynucleotide (or DNA) was plotted as a function of concentration of added polynucleotide. Fig.4 shows a representative plot for the PPA-DNA interaction. The concentration of DNA corresponding to the mid-point of the spectrophotometric titration follows the order poly(dG-dC) > c.t. DNA > poly(dA-dT), suggesting a reverse order for the affinity constants for the interaction of these polynucleotides with PPA. It may be noticed from the figures that there is very little change in the absorbance of PPA due to its binding with poly(dG-dC). Similar results were obtained for PAP but are not shown here. (ii) The molar ellipticity value corresponding to the peak of the induced band in the CD spectra of the ligand-DNA complexes was plotted as a function of r', the ratio of the concentrations of the added ligand and DNA. A series of representative curves for

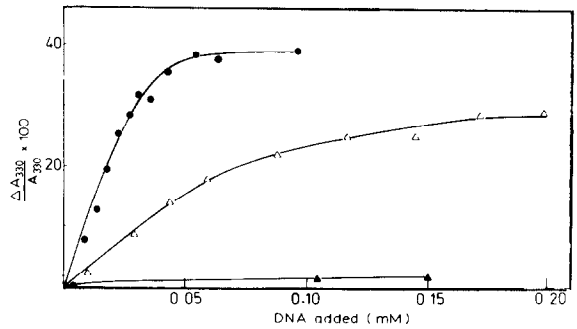

Fig.4. Percent increase in absorption at 330 nm for PPA (10 μM) as a function of r for different DNAs: poly(dA-dT) (—●—), c.t. DNA (—△—) and poly(dG-dC) (—▲—).

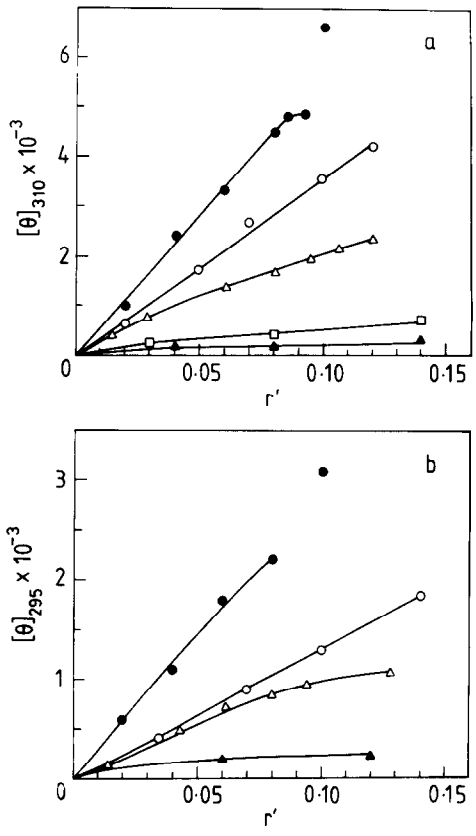

Fig.5. Variation of molar ellipticity, $[\theta]$, with r' for the complexes of (a) PPA and (b) PAP with different DNAs: poly(dA-dT) (—●—), c.p. DNA (—○—), c.t. DNA (—△—), m.l. DNA (—□—) and poly(dG-dC) (—▲—). Note that (both for PPA and PAP) in the case of poly(dA-dT), one point in the titration curve lies above it. In analogy to Nt [8], such a deviation from the titration curve can be ascribed to a second mode of binding.

such spectropolarimetric titrations for the PPA-DNA interaction is shown in fig.5a. The slopes of these curves indicate that the affinity constants follow the order poly(dA-dT) > *Clostridium perfringens* (c.p.) DNA > c.t. DNA > *Micrococcus lysodeikticus* (m.l.) DNA > poly(dG-dC). In fact, the intensity of the induced band in the case of poly(dG-dC) is very small. The spectropolarimetric titration curves for the PAP-DNA interaction are shown in fig.5b. The slopes of the curves follow the same order as observed in the case of PAP.

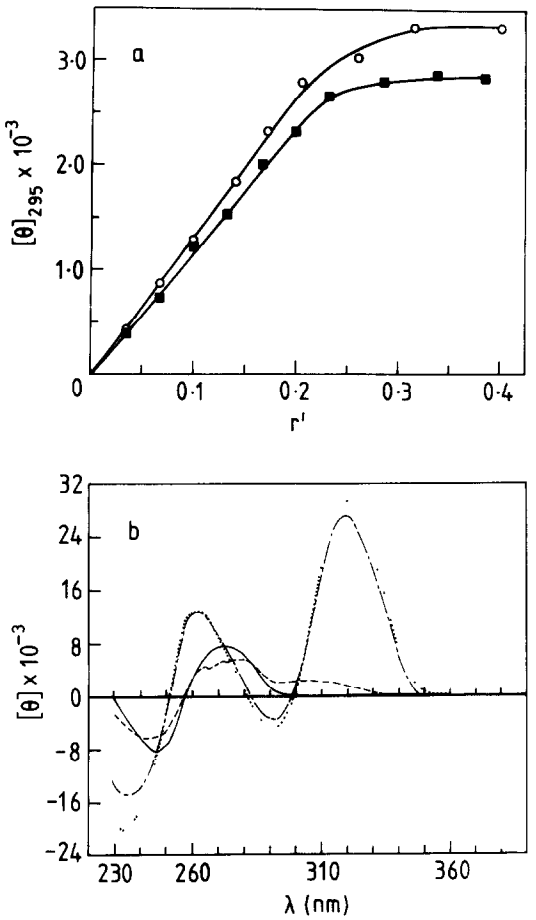

Fig.6. Evidence for minor groove binding of PAP and PPA. (a) Variation of $[\theta]_{295}$ with r' for the complexes of PAP with c.p. DNA (—○—) and T_4 DNA (—■—). (b) CD spectra of c.t. DNA (400 μM) (——), its complex with PPA (r' = 0.1) (– – –), and on addition of Dst (r' = 0.1) (-——-) to the above complex. The CD spectrum of c.t. DNA (400 μM) in the presence of Dst (r' = 0.1) (···) is also shown for comparison.

These results indicate that these synthetic analogs exhibit AT-base specificity akin to that reported for Dst [5].

3.3. *Minor groove binding*

The minor groove binding nature of these ligands could be suggested from the following two experiments. The affinity of the ligands for c.p. and T_4 DNAs was compared by means of spectropolarimetric titrations. Fig.6a shows that there is no significant difference in the binding affinities for PAP towards the two DNAs, as indicated from the slopes of the titration curves. Thus the blockage of the major groove by bulky glucose residues in the T_4 DNA [6] has not influenced its affinity for PAP. Similar results were obtained for PPA. In the other experiment, Dst, a well-established minor groove binding agent, was added to an equilibrium mixture of PPA and c.t. DNA. As evident from fig.6b, addition of Dst gives rise to a CD spectrum characteristic of the Dst-DNA complex. This suggests that Dst has replaced PPA from its minor groove binding site. Similar type of changes in the CD spectra were observed when Dst was added to an equilibrium mixture of PAP and c.t. DNA (not shown).

4. CONCLUSION

The above studies indicate that neither of the Dst analogues, PPA or PAP, have lost AT-base specificity due to the substitution of an *N*-methylpyrrole unit by β-alanine. One might, therefore, suggest that the delocalisation of electrons (by the presence of consecutive pyrrole-carboxamide units) does not play a significant role in determining the base specificity of binding of Dst to DNA. It may then be suggested that the delocalisation contributes only to the stabilisation of the drug-DNA complex. Our results (communicated) on the comparative binding affinities of PPA and PAP towards poly(dA-dT), a polynucleotide with a B-DNA structure [9], indicate that the binding constants depend on the number and relative positions of the *N*-methylpyrrole groups. The present study supports the prevailing view that the isohelical geometry of the ligands and the proper orientation of their hydrogen bonding loci gives rise to the observed base specificity [2]. It is worth mentioning that a

synthetic analog of Dst containing three benzene rings in place of the pyrroles of Dst (leading to an increased curvature of the ligand backbone) exhibits less pronounced AT-base selectivity, compared to Dst [7]. Presently we are investigating the antibiotic properties of these analogs.

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