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# Interaction of non-intercalative drugs with DNA: Distamycin analogues*

MALINI RAJAGOPALAN, JAYALEKSHMY AYYER and
V. SASISEKHARAN†
Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India.

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**Abstract.** Distamycin and netropsin are two oligopeptides which bind to DNA in a non-intercalative manner. Analogues of distamycin have been synthesized and their binding with poly d(A-T) studied using ultraviolet absorption spectroscopy. Preliminary biological activity tests on a gram positive bacteria using these analogues have also been carried out.

**Keywords.** Non-intercalative binding; DNA; distamycin analogues

## Introduction

The antitumour activity of a number of naturally occurring as well as synthetic compounds has been demonstrated although their mode of action remains largely unknown. The oligopeptide antibiotics, netropsin and distamycin bind in a non-intercalative mode to B-DNA (Zimmer, 1975). These molecules are composed of pyrrole rings joined by peptide units. They show antibacterial, antiviral and antitumour properties (Zimmer, 1975) in some systems as well as inhibition of DNA synthesis *in vitro* (DiMarco *et al.,* 1964). They have unusually high affinity for A-T rich regions (Zimmer, 1975; Luck *et al.,* 1974) and it is believed that hydrogen bonds are formed between O2 of thymine and N3 of adenine with N–H atoms of the peptide backbone (Zasedatelev *et al.,* 1976; Berman *et al.,* 1979). Though it has not been conclusively proved, it is generally believed that the DNA binding property is responsible for the biological activity of these molecules.

From an examination of the crystal structures of netropsin (Berman *et al.,* 1979; figure 1 a) and distamycin analogues (Gurskaya *et al.,* 1979; figure 1b) it is readily seen that these molecules prefer bow-shaped structures with curved backbone which places the amido N-H groups in favourable positions for hydrogen bonding to the DNA base atoms mentioned above. Model building studies have shown that with small torsional changes from the crystal structures, these molecules attain geometrical shapes which would enable them fit well in the minor groove of B-DNA (Chandra *et al.,* 1972; Kolchinskii *et al.,* 1975). Therefore, the design of any molecule with binding capacity

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† To whom reprint requests should be addressed.

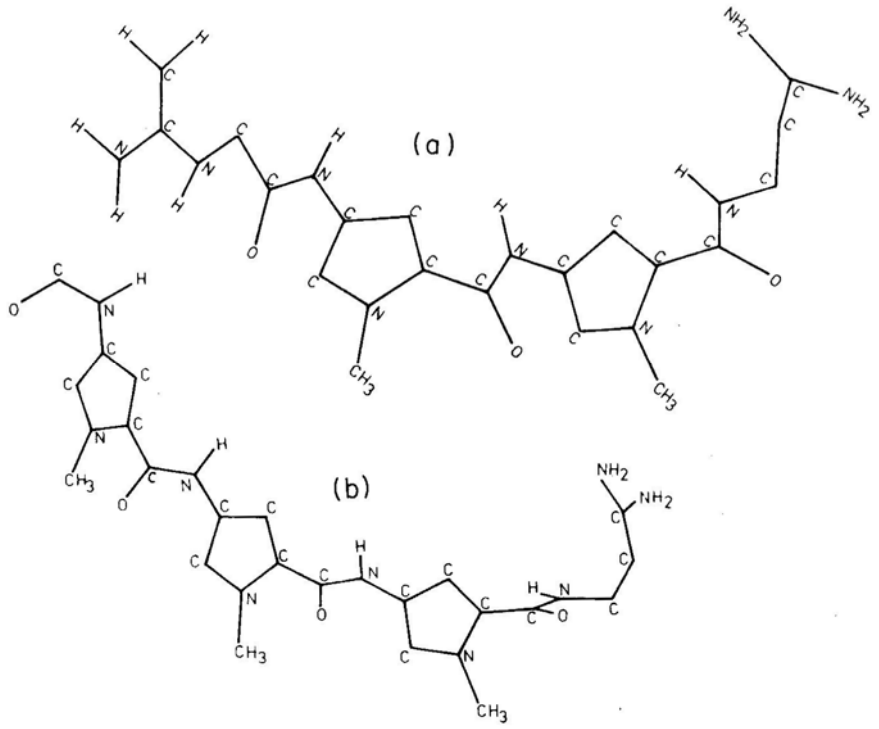


**Figure 1.** (a) A projection of the structure of the netropsin molecule. (b) A projection of the structure of the distamycin molecule.
Note in the above two molecules, the projection axes are the same to show the curvature of the backbone which fits into the minor groove of B-DNA.

and specificity close to that of netropsin and distamycin requires a certain amount of curvature in the molecule itself, so as to have a stereochemical fit with DNA.

To investigate the role of this curvature in the binding and biological properties of such molecules, a novel distamycin analogue was synthesized. N-methyl pyrrole rings of distamycin were replaced by 1,3 disubstituted benzene rings. This increases the inherent curvature in the molecule as compared to netropsin or distamycin (Berman *et al.,* 1979; Gurskaya *et al.,* 1979). Bis and tris (*m*-benzamido) compounds prepared by us (figure 2) are expected to bind to DNA in a similar fashion as the distamycin class of drugs (Zimmer *et al.,* 1972). Binding studies of bis, and tris (*m*-benzamido) compounds indeed show that the latter binds to poly d(A-T) and causes a significant elevation of the melting temperature. This communication contains some of the preliminary results obtained using the above compounds.

## Materials and methods

Poly d(A-T) (sodium salt) used for the binding studies was purchased from Boehringer Mannheim (Lot No. 1443325). The bis and tris (*m*-benzamido) compounds were

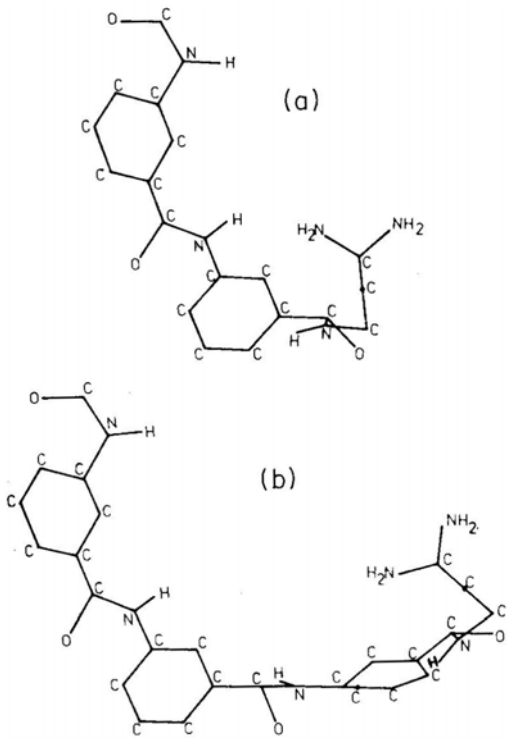


**Figure 2.** (a) A projection of the structure of the bis (*m*-benzamido) compound, (b) A projection of the structure of the tris (*m*-benzamido) compound.
The same projections as in figure 1a and 1b have been shown to indicate the curvature.

synthesized in this laboratory following a procedure similar to that employed for the synthesis of distamycin A (Bialer *et al.,* 1978). Infra-red and nuclear magnetic resonance spectroscopy were used for characterising these compounds. The solution (1 mM) of the bis (*m*-benzamido) compound was prepared in water and that of tris (*m*-benzamido) compound was prepared in 1:10 ratio of methanol: water mixture.

Ultra-violet spectroscopy studies were carried out using Beckman DU-8B spectrophotometer. The reference quartz cell had 20 mM NaCl and the sample quartz cell contained the poly d (A-T) solution. Different volumes of the bis (*m*-benzamido) and the tris (*m*-benzamido) compounds were added to 1 ml each of the 20 mM NaCl and the poly d(A-T) solutions (in reference and sample cell respectively) corresponding to the ratios of substrate: poly d(A-T) of 0.1:1, 0.2:1 and 0.5:1 respectively. The peptide solution (same volume) was added to both the reference and sample cells, so as to monitor only the effect due to complexation of poly d(A-T) with the substrates. The formula:

$$\frac{\text{Absorbance calculated} - \text{absorbance observed}}{\text{Absorbance calculated}} \times 100,$$

gives the percentage change in absorption at different substrate: poly d(A-T) ratios. The absorbance calculated is obtained by incorporation of the volume correction due to the addition of peptide solution to the fixed volume of the 20 mM NaCl/Poly d(A-T).

Melting temperature studies were also carried out with different ratios of the substrate to poly d(A-T) as in the ultra-violet spectroscopy studies. The temperatures were scanned from 20°-80°C. Freshly prepared solutions of the bis and tris (*m*-benzamido) compounds were used for each experiment, as there was found to be loss of absorbance with time. Different volumes of these peptide solutions corresponding to ratios of 0·1: 1 and 0·2:1 of substrate to poly d(A-T) were added to the sample cells containing poly d(A-T).

Biological activity tests were carried out on a Gram positive bacteria (*Staphylococcus aureus*) with both the bis and tris (*m*-benzamido) compounds by dilution test method.

*Results*

Results of the ultra-violet absorption spectroscopy studies on the bis and tris (*m*-benzamido) compounds with poly d(A-T) are shown in figure 3 at the different ratios of

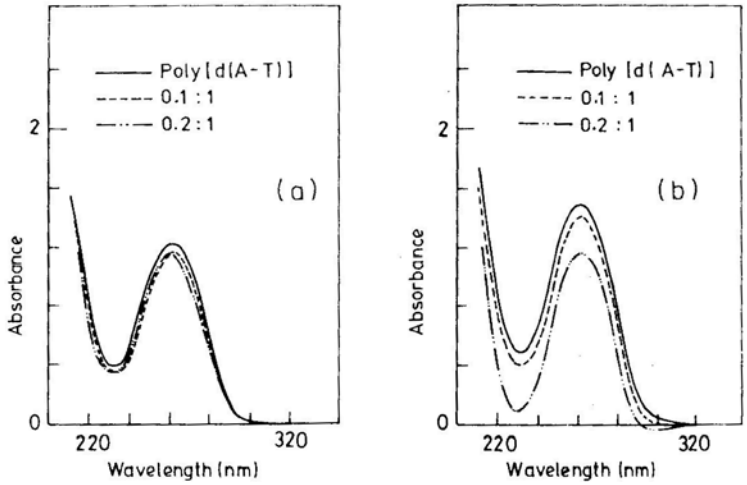


**Figure 3.** (a) Ultra-violet spectra of poly d(A-T) and the poly d(A-T) complexed with the bis (*m*-benzamido) compound for the ratios indicated, (b) Ultra-violet spectra of poly d(A-T) and the poly d(A-T) complexed with the tris (*m*-benzamido) compound for the ratios indicated.

0·l: 1, 0·2:1 and 0·5; 1 of the substrate: poly d(A-T). It may be noted that there is a significant hypochromicity at 261 nm in the ultra-violet absorption spectra of the tris (*m*-benzamido): poly d(A-T) complex as compared to poly d(A-T) at the different ratios. A similar but less pronounced effect is seen for the bis (*m*-benzamido); poly d(A-T) complex. The hypochromic change observed for the tris complex is about 22% as compared to about 7 % for the bis complex at the 0·2:1 ratio. The hypochromic change observed (not shown) for 0·5:1 ratio is 30% for the tris complex and 8 % for the bis complex. A small red shift was also observed in the $\lambda_{max}$ of poly d(A-T) on complexation. $\lambda_{max}$ of poly d(A-T) at 261·7 nm shifted to 262·5 nm on addition of the tris (*m*-benzamido) compound. The melting profiles of poly d(A-T) and its complexes

with the bis (*m*-benzamido) and tris (*m*-benzamido) compounds are shown in figure 4a and 4b respectively for the 0·1:1 and 0·2:1 ratios. The melting/transition temperature ($t_m$) of poly d(A-T) alone is at 47°C whereas for the bis (*m*-benzamido) poly d(A-T) complex the $t_m$ is at 52°C indicating a 5°C increase in the transition temperature of poly d(A-T) on complexation with the bis (*m*-benzamido) compound. On the other hand, it is seen from figure 4b that the $t_m$ changes from 47°C (poly d(A-T) alone) to 60°C on complexation with the tris (*m*-benzamido) compound at 0·2:1 ratio showing a 12°C increase in the transition temperature of the tris (*m*-benzamido): poly d(A-T) complex as compared to the uncomplexed poly d(A-T).

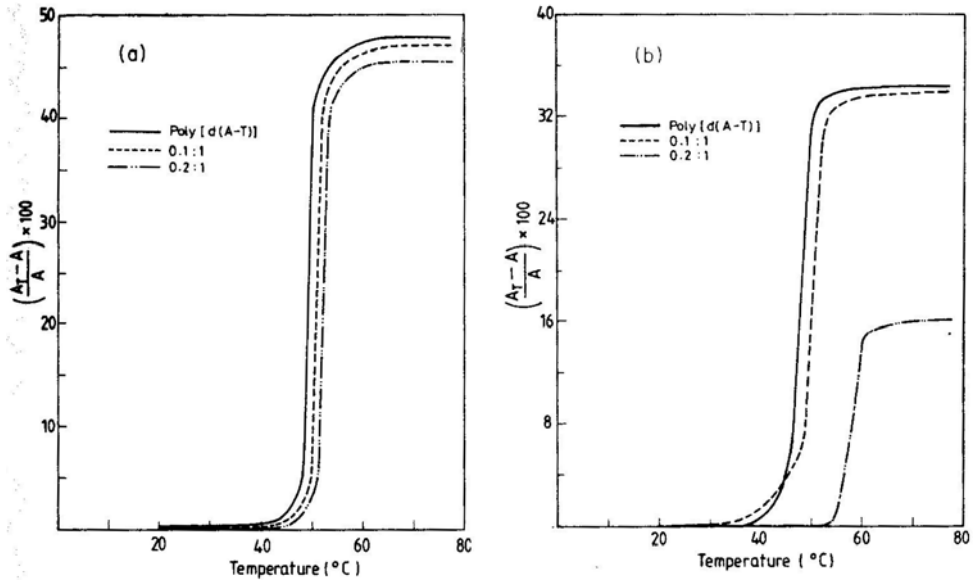


**Figure 4.** (a) Melting profiles of poly d(A-T) and the poly d(A-T) complexed with the bis (*m*-benzamido) compound for the ratios indicated. (b) Melting profiles of poly d(A-T) and the poly d(A-T) complexed with the tris (*m*-benzamido) compound for the ratios indicated.

Preliminary biological activity tests performed on the Gram positive bacteria *S. aureus* by the dilution test method show that there is a zone of inhibition with the tris (*m*-benzamido) compound at concentrations as low as 50 $\mu$g as compared to the bis (*m*-benzamido) compound which showed no zone even at 200 $\mu$g. Under the same experimental conditions, distamycin A showed a zone of inhibition only at a concentration of 125 $\mu$g. These tests are indicative of antibacterial activity of the tris (*m*-benzamido) compound. Details of these will be published elsewhere.

## Discussions

The antibacterial activity studies show some interesting results even though the preliminary results mentioned above do not establish the comparative strength and specificity of binding of these (*m*-benzamido) compounds and netropsin and distamycin. The tris (*m*-benzamido) compound is shown to be more active than

distamycin A from the biological activity tests carried out on *S. aureus.* Although, a number of similar compounds were reported earlier in the literature by several workers (Atwell and Cain, 1968; Atwell *et al.,* 1968a,b; Cain *et al.,* 1968, 1969; Jones and Wooldridge, 1968), there are no reports on the bis and tris (*m*-benzamido) compounds investigated and described in this communication. Earlier studies were mostly carried out with compounds containing benzene rings linked by peptide units in the *para* positions and therefore do not have the curvature similar to that of netropsin and distamycin. On the other hand, the *meta* linkage provides curvature to the molecule as already described (figure 2a and 2b). The preliminary results obtained by us call for a renewed thrust in the synthesis of such molecules using conformational principles to assure binding to DNA. It is well known that use of distamycin and netropsin as antimicrobial agents were hampered by their cytotoxicity. Designing of drugs with similar geometrical, electrostatic and Van der Waals' features as the distamycin class but with distinct and carefully controlled alterations such as the one described here may help find newer and better drugs.

## Acknowledgements

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