

# Mono-, di- and trimeric binding of a bis-netropsin to DNA

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**Abstract** An unusual 3:1 stoichiometry for complex formation between an elongated bis-netropsin compound and its binding site on DNA has been observed. Circular dichroism measurements distinguish two types of complexes formed between this bis-netropsin and poly[d(A-T)]·poly[d(A-T)]. The first type is characterized by a 1:1 saturating ratio of bound molecules per ten base pairs. Formation of the second type results from the cooperative binding of two additional bis-netropsin molecules to the first type of complex. In contrast to these results observed for binding to the alternating polynucleotide, only the 1:1 type of complex is formed when this ligand binds to the homopolymer poly(dA)·poly(dT).

**Key words:** DNA/drug interaction; Minor groove binding; Side-by-side binding; Circular dichroism

## 1. Introduction

The antiviral and antitumor antibiotics netropsin and distamycin A, as well as their derivatives, such as bis-netropsins, were the first ligands for which localization in the minor groove upon binding to DNA was predicted, along with the underlying molecular mechanism of A·T-specificity (for review see our original papers [1–4], confirmed later by NMR and X-ray studies [5,6] in the case of netropsin and distamycin A binding). Initially it was assumed that each binding site can be occupied by only one bound ligand molecule, due to the narrow width of the DNA minor groove.

The side-by-side dimeric motif for binding two ligand molecules in the DNA minor groove was first discussed for netropsin binding to poly[d(A-T)]·poly[d(A-T)] by Zimmer and coworkers [7], based on physico-chemical studies using circular dichroism (CD) measurements. Subsequently, this mode of DNA–ligand interaction was confirmed by Wemmer et al. [8] in NMR studies of distamycin–oligonucleotide complexes. Enabling NMR studies of netropsin, distamycin A and their analogs bound to various double-stranded oligodeoxyribonucleotides [9] led Dervan et al. [10] to the clever design of a new type of DNA-specific ligand capable of forming intramolecular dimers in the DNA minor groove. These ligands were composed of pyrrolecarboxamide backbones linked by flexible oligomethylene chains of different length which facilitate the formation of an antiparallel, side-by-side dimeric motif localized in a slightly widened minor groove. More recently, Bruice and

coworkers [11] have proposed an additional binding mode for distamycin A, based on NMR studies, involving a complex of four bound ligands localized in one region of the minor groove.

In this paper, the ability to form the side-by-side motif in the DNA minor groove was investigated using a bis-netropsin compound containing *N*-propylpyrrole instead of *N*-methylpyrrole rings. We observe that an additional two bis-netropsin molecules bind per ten base pairs of poly[d(A-T)]·poly[d(A-T)] already occupied by one bound ligand, thereby forming a novel 3:1 complex not previously reported. Formation of this trimeric complex is a cooperative phenomenon quantitatively described and analyzed in this study.

## 2. Materials and methods

Bis-netropsin (see Fig. 1) ( $\epsilon_{305} = 40,000$ ) was synthesized and purified as described earlier [4]. Poly(dA)·poly(dT) ( $\epsilon_{260} = 12,000$ ) and poly[d(A-T)]·poly[d(A-T)] ( $\epsilon_{260} = 13,600$ ) were obtained from P.L. Biochemicals (USA). All polynucleotides were used as supplied, without further purification. Polynucleotides were dissolved in 1 mM phosphate buffer (pH 6.0) containing 0.1 mM EDTA, dialyzed for 6 h against 0.06 M phosphate buffer (pH 6.0) containing 0.5 mM EDTA and finally dialyzed for 24 h against 0.06 M phosphate buffer (pH 6.0) in the absence of EDTA. All binding experiments were carried out in 0.06 M phosphate buffer (pH 6.0) at 20°C in the presence or absence of an additional 0.3 M NaCl.

CD spectra were obtained with a Jobin-Yvon Mark III dichrograph (France). Concentration of the polymers was approximately 0.1 mM in base pairs (bp). Derivative CD spectra, with respect to added ligand concentration, were obtained by subtracting subsequently measured spectra and dividing this difference by the difference in added ligand concentration, which did not exceed 0.001 mM.

## 3. Results and discussion

The binding of bis-netropsin to poly(dA)·poly(dT) (Fig. 2a) and poly[d(A-T)]·poly[d(A-T)] (Fig. 2b) is accompanied by significantly different spectral changes. In the case of poly(dA)·poly(dT), the maximum in the CD band in the 300–350 nm range occurs at 315 nm with a maximal CD absorbance value of  $\Delta\epsilon_{315} = 10$  per mole of base pairs. Binding to poly[d(A-T)]·poly[d(A-T)] is accompanied by more complicated spectral changes characterized by a shift of the maximum from 315 nm to 328 nm, as shown by the arrows in Fig. 2. In this case, the maximal amplitude of molar absorbance corresponds to  $\Delta\epsilon_{328} = 36$ .

In order to estimate the CD spectral modes characterizing the binding process at different ligand to DNA base pair ratios, (C/bp), we have obtained derivative CD spectra with respect to the concentration of bis-netropsin. These derivative CD spectra, presented in Fig. 3, clearly demonstrate the existence of two

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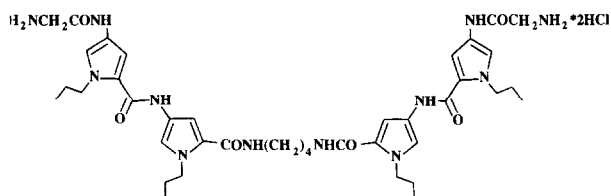


Fig. 1. Chemical structure of bis-netropsin.

different spectral bands, most probably attributable to different types of ligand–DNA interactions.

In contrast, binding to poly(dA)·poly(dT) (Fig. 3a) is accompanied by only one type of spectral band with a maximum at 313 nm. The same type of spectral band is observed in the initial stage of the titration of poly[d(A-T)]·poly[d(A-T)] by the ligand, corresponding to  $C/bp < 0.07$  (see curve 1 in Fig. 3b). On subsequent titration of the alternating polymer in the range  $0.13 < C/bp < 0.17$ , the second type of spectral band can be estimated (curve 2 in Fig. 3b). The corresponding spectral curve goes through zero at 312.5 nm and has a maximum at 328 nm. The isosbestic point for curves 1 and 2 occurs at 317.5 nm.

Separation of the CD spectra, based on the derivative spectral representation, makes it possible to observe the formation of the first type of complex alone using a titration plot at 312.5 nm, where the second type of the complex does not contribute to the CD signal. On the other hand, observation of the second type of complex is simplified by using titration data at 340 nm, where the contribution of corresponding spectral mode is about 5 times greater than the contribution of the first spectral mode. Both characteristic wavelengths as well as the isosbestic point are marked in Fig. 3 by dotted lines.

Titration plots for both double stranded alternating and homopolymers carried out at 312.5 nm, 317.5 nm and 340 nm are presented in Fig. 4. The intersection of lines delineating the initial slopes and saturation levels, marked by dotted lines, shows that, in the case of binding to poly(dA)·poly(dT), only the first type of complex is observed, characterized by

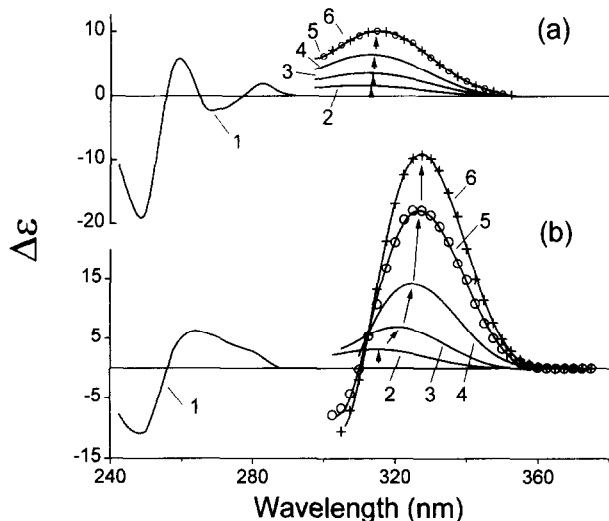


Fig. 2. CD spectra of bis-netropsin bound to poly(dA)·poly(dT) (a) and poly[d(A-T)]·poly[d(A-T)] (b).  $\Delta\epsilon$  = circular dichroism amplitude per mole of base pairs. Ratios of ligand added with respect to DNA base pairs ( $C/bp$ ) are: (a) 0 (1), 0.018 (2), 0.045 (3), 0.09 (4), 0.18 (5), 0.45 (6); (b) 0 (1), 0.051 (2), 0.10 (3), 0.16 (4), 0.26 (5), 0.51 (6).

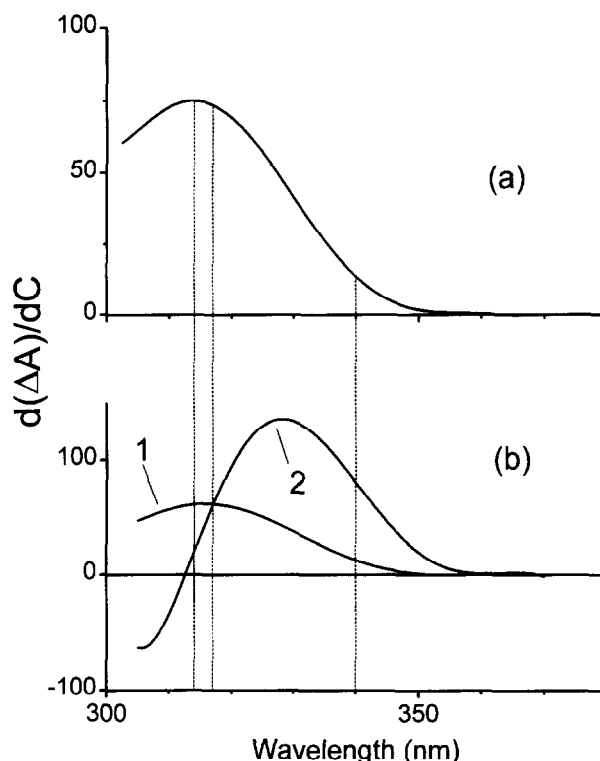


Fig. 3. Derivative spectra of circular dichroism amplitude ( $\Delta A$ ) with respect to concentration ( $C$ ) of bis-netropsin added to poly(dA)·poly(dT) (a) and poly[d(A-T)]·poly[d(A-T)] (b); curve 1 =  $C/bp < 0.07$ , curve 2 =  $0.13 < C/bp < 0.17$ .

$C/bp = 0.1$  (with an accuracy of about 0.02). This means that this elongated bis-netropsin molecule binds to the poly(dA)·poly(dT) minor groove, covering about one turn of the double helix (in agreement with our previous results [4]).

As can be seen from Fig. 4b, the first type of complex characterized by  $C/bp = 0.1$  or 1:1 ratio of bound molecules to ten base pairs (see curve 1) is also formed when bis-netropsin binds to poly[d(A-T)]·poly[d(A-T)]. Furthermore, this type of complex persists in the presence of the second type of the complex. Formation of the second type of complex between bis-netropsin and the alternating polymer (see curve 2 in Fig. 4b) appears to be a cooperative phenomena which begins only in the presence of the first type of complex and is complete at  $C/bp = 0.3$ . Thus total complex formation requires 3 bound molecules per 10 base pairs. As is clearly evident from Fig. 4b, formation of the second type of complex takes place at  $0.1 < C/bp < 0.3$ . Hence it requires an additional ligand molecule per five base pairs, or two molecules per ten base pairs, of DNA already occupied by a molecule bound in the first type of complex. Curve 3 in Fig. 4b shows a titration plot at 317.5 nm (isosbestic point) simply indicating total formation of the complex characterized by a 3:1 ratio of bound ligand to binding site composed of ten base pairs of poly[d(A-T)]·poly[d(A-T)]. Formation of the 3:1 complex was not affected by ionic strength up to 0.3 M NaCl.

The similarity between the CD spectra for the complex formed with poly(dA)·poly(dT) and the first complex formed with poly[d(A-T)]·poly[d(A-T)], in the 300–350 nm wavelength range, suggests that these two complexes involving different

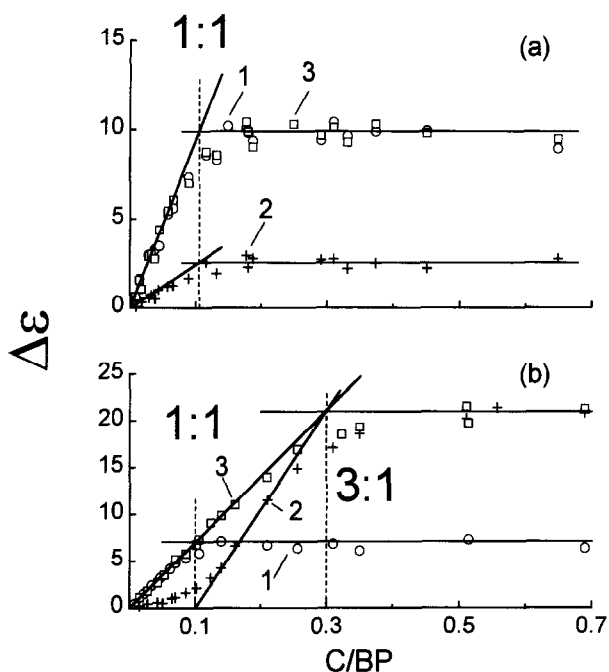


Fig. 4. Titration of poly(dA)·poly(dT) (a) and poly[d(A-dT)]·poly[d(A-dT)] (b) with bis-netropsin at 312.5 nm (curve 1, ○), 317.5 nm (curve 2, +) and 340 nm (curve 3, □).

polynucleotides are closely related. This similarity is further supported by the small, quantitative difference between the  $\Delta\epsilon_{312.5}$  induced per bound ligand between these two complexes. A similar comparison involving the second complex formed with the alternating polynucleotide is difficult, as the spectrum undergoes a significant shift to higher wavelength.

Until now only monomeric [1–4], dimeric (side-by-side) [7–9] and tetrameric [11] types of complexes formed between minor groove binding ligands and DNA have been described, based on NMR or X-ray data. We have not only observed trimeric binding behavior with the bis-netropsin molecule containing four methylene groups in the linker region (Fig. 1), but also with the closely related ligand containing five methylene groups in the linker (data not shown). Observation of a 3:1 or trimeric type of complex between bis-netropsins and poly[d(A-T)]·poly[d(A-T)] increases the known possibilities for the binding of pyrrolecarboxamide-containing ligands to DNA.

Data presented in Fig. 4b indicate that the novel trimeric type of complex described in this paper consists both of monomeric and dimeric types of complexes. Thus it appears that some

NMR data obtained earlier for the dimeric type of complexes may also be relevant to the trimeric complexes, as there may be contacts between ligand and DNA (in the first type of the complex) and also between the pair of the ligands (in the second type of the complex).

At this point, one can speculate on the geometry of the trimeric complex. One possible geometry involves three ligands bound side-by-side, which would require a considerably enlarged minor groove. Alternatively, the second complex may involve a side-by-side dimer bound on the exterior of the minor groove. Another binding mode could involve two folded ligands, each interacting with half of the initially bound ligand. Alternatively, it is also possible that the dimer, representing the second type of complex, binds in the major groove, across from the first bound ligand. Additional experiments are needed in order to develop a molecular model for this new trimeric complex between minor groove binding ligands and DNA.

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